ESSENTIALS OF LABORATORY DIAGNOSIS

FAUGHT



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ESSENTIALS

OF

LABORATORY DIAGNOSIS

DESIGNED FOR
STUDENTS AND PRACTITIONERS

BY

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PREFACE TO FIFTH EDITION.

In presenting this the "Fifth Edition" of "Essentials of Laboratory Diagnosis" to the profession, the author desires it clearly understood that in the re-arrangement accompanying this complete revision, it has been found necessary to eliminate wherever possible all discussion of clinical pathology, and to confine the subject matter more closely to laboratory technic.

The same rule as heretofore applied to the selection of material has been employed, so that frequent parrallelism between methods has been avoided whenever possible, in an effort to avoid undue enlargement of the book, thereby endeavoring to maintain its function as a concise and compact laboratory manual.

F. A. F.

PREFACE TO FIRST EDITION.

A KNOWLEDGE of so many branches of medicine is required of the medical student of to-day, and as the time at his command is comparatively limited, a manual embodying the essentials of the subject such as this aims to do cannot but prove useful. At the same time, it is believed to contain all information necessary to provide a working outline of clinical laboratory methods for the busy practitioner.

The book is not intended to take the place of the many excellent and exhaustive text-books on Clinical Medicine, but rather to supplement them, by pointing out to the busy student and practitioner simple and reliable methods by which he may obtain the information desired, without unnecessary expenditure of valuable time upon difficult, tedious or untried methods.

The author has endeavored in this work to present in as concise a manner as possible a selection of the analytical methods employed in the clinical laboratory, without burdening the reader with unnecessary detail, cumbersome methods, etc., many of which are extremely difficult, often requiring considerable knowledge of general chemistry and elaborate apparatus, which may place them beyond the reach of the practitioner.

For these reasons it is hoped that this little work will prove equally valuable to the student and the practicing physician.

In preparing this work special effort has been made to bring the subjects treated up to date, by the introduction of such new methods as have proven reliable. Some of the

material is entirely new, and many of the plates and cuts have been prepared from original drawings and photographs by the author.

The appendix has been arranged to furnish a working basis for the equipment of a clinical laboratory, at the same time affording reference for the preparation of stains, reagents, etc., mentioned in the text.

The leading authorities have been freely consulted, and much material has been obtained from such authorities as v. Jaksch, Sahli, Caillé, Grawitz, Krehl, Max Braun, Tyson, Abbott, Purdy, Remsen, and Holland.

The author takes this opportunity to express his appreciation of the many valuable suggestions received from Drs. Judson Daland and Wm. Egbert Robertson, and Mr. Geo. B. Johnson, of the F. A. Davis Company; also, to his associate, Dr. Francis J. Dever, for invaluable aid in the preparation of the manuscript and in correcting the proof.

F. A. F.

1831 Chestnut Street,
Philadelphia.

INTRODUCTORY NOTE.

By Judson Daland, M.D.
Professor of Clinical Medicine, Medico-Chirurgical College, Philadelphia.

This book should be in the possession of every medical student and most practicing physicians. It contains, as its title implies, the essentials, and only the essentials of those procedures necessary to clinical laboratory diagnosis. The peculiar value of this book resides in the concise and practical manner in which each subject is treated and each test described, and the entire absence of all superfluous data.

This book is particularly well suited, not only to the medical student in the preparation of cases assigned to him for study, but also to practicing physicians, who have grasped the necessity of establishing a small laboratory in connection with every-day work. Those possessing such a laboratory will find it necessary to consult this book at short intervals. The more it is employed the more will its practical value be demonstrated. I unhesitatingly recommend this work to medical students, and trust that it will find a place in the laboratory of every practicing physician.

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I.

THE MICROSCOPE.

ITS ACCESSORIES AND MICROSCOPIC TECHNIC.

SELECTION OF THE INSTRUMENT.

In purchasing a microscope one should consider nothing but the best. This is true economy, for a cheap or inferior instrument is always an unsatisfactory one, which, when once bought, is hard to get rid of, and if later discarded for a better instrument, represents a total loss. The possession of a good microscope is an absolute essential. Its services are required in almost every investigation of modern clinical diagnosis, and there is hardly a chapter in this work which does not call for its use. While the finest and most expensive instruments are still of foreign make, there are, nevertheless, many of American make which compare favorably with the imported instruments. An example of a good American instrument is shown in Fig. 1, and is one of a series manufactured by the Bausch and Lomb Optical Co.

CARE OF THE MICROSCOPE.

In handling any instrument it is necessary to see that it is always grasped by some one of its solid parts—the base or the standard. Some of the newer makes are provided with an aperture in the standard for this very purpose.

If the instrument is to be in daily use it should be kept under a bell jar or in a specially prepared cabinet built above the work table. Reference to Fig. 1 will illustrate the mechanism and the different parts with which one should become familiar before attempting to use the instrument. Efforts to use the microscope by any one unfamiliar with its different parts and adjustments should never be permitted, since careless handling may result in serious damage to the objective or other delicate parts.

DESCRIPTION OF THE MICROSCOPE.

Referring to Fig. 1, the various parts of the instrument about to be described may be located.

The Ocular, or Eye-Piece, consists of one or more converging lenses, the combined action of which is to magnify the

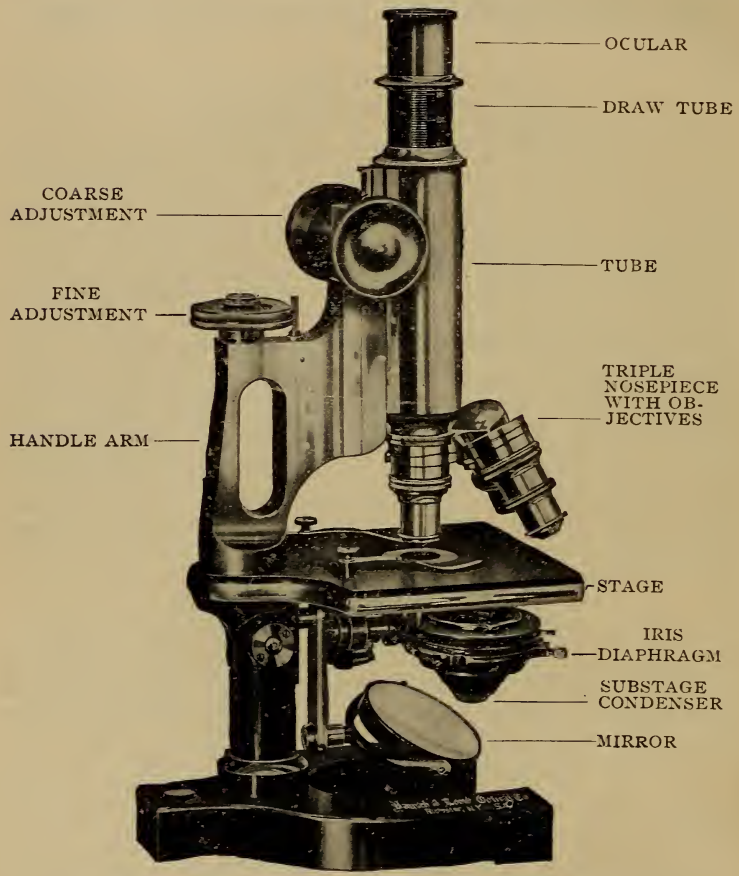


FIG. 1.—AMERICAN-MAKE MICROSCOPE SHOWING TRIPLE NOSEPIECE AND APERTURE IN STANDARD TO FACILITATE HANDLING.

image formed by the objective. This part is contained in a tube of its own, which telescopes into the top of the barrel, and is the part which the eye approaches when viewing the object.

Various oculars are usually provided, and are numbered from 1 to 8. As the number advances, the magnifying power of the ocular increases; at the same time the length of the tube decreases. It is advised, when increased magnification is desired, to accomplish this by increasing the power of the objective rather than by using stronger oculars. The clearness and definition of the picture will then be preserved, whereas if the power of the ocular is increased it is usually at the expense of definition.

It will be found of great convenience to cement into one of the oculars a fine hair or eyelash. This should rest upon the upper surface of the central diaphragm of the ocular, when it will always be in focus. This serves as a pointer which can be used to single out from a blood or other specimen some special point of interest that it is desired to demonstrate to another person. This is particularly valuable in class demonstration.

The Barrel, or Tube, is the large tube of the microscope, which serves as a conductor of the rays as they pass from the objective to the ocular.

The Objective consists of a system of converging lenses arranged at the lower end of the barrel, where it forms a magnified inverted image of the object. Upon this piece depends the magnifying power of the microscope. Most objectives are designated by numbers running from 1 to 15, these numbers representing the fractions of an inch at which the lens operates. Foreign makes are designated by letters which are not in any way directly comparable to the numbers of the American objectives. For one desiring three objectives, the most useful numbers will be found to be the 3, the 7 or 6, and the 12-oil-immersion, the 3 and 6 or 7 being chiefly employed in blood-counting, while the 12th is necessary for blood-examination and bacteriologic work.

The Stage is the table fastened below the barrel and in a right-angle plane to it. This serves to retain the object in a vertical plane to the optical axis of the instrument. The table is provided with spring clips to better hold the slide in position during examination. The newest Leitz scope is furnished with a movable (not mechanical) stage, which, by the operation of two milled screws, is capable of slight movement in all direc-

tions. This, when a mechanical stage is not at hand, is of great service in searching blood and bacteriologic slides.

The Reflector is a small mirror situated below the stage which serves to direct the rays of light upward through the object in the optical axis of the microscope. The reflector has two sides, one carrying a concave, and the other a plane, mirror.

The Sub-Stage Condenser (Abbe's) consists of a system of lenses arranged between the stage and the reflector. The object of this part of the instrument is to collect and condense the rays as they come from the reflector, so that they are focused upon the object, thus furnishing brilliant illumination.

The Iris Diaphragm is now universally employed for controlling the intensity of the illumination. This is held in the same carrier as the condenser, and is just below it. By means of a lever every gradation of light, from the most intense to absolute darkness, is readily obtained. The proper manipulation of this diaphragm constitutes a very important part of the practical knowledge gained from the use of the microscope, and has much to do with the success of many investigations.

The Adjustments.—The *coarse* adjustment is the rack-and-pinion mechanism projecting from the upper part of the standard, and is employed to rapidly raise and lower the barrel and its attachments. The *fine* adjustment is a micrometer screw situated usually below the rack and pinion. This serves the purpose of very gradually raising or lowering the barrel in order to obtain exact focus.

The Draw-Tube is a very important adjunct of high-grade instruments, because by its skilful manipulation slight errors in refraction, due to inequalities in slide or cover-glass, may be corrected.

The Nose-Piece, or collar, is fastened to the lower end of the barrel, and permits the attachment of two or three objectives at one time in such a position that, by rotation of the collar, any one of them may be immediately brought into the axis of the instrument.

The most important accessories of the microscope are the objectives, and the quality of all microscope work largely depends upon their perfection. While the quality of objectives vary much, one cannot go far astray if they are obtained through

a reputable supply house or from a well-known manufacturer, such as the Zeiss or Leitz abroad, and the Bausch and Lomb Optical Co., U. S. A.

THE OIL-IMMERSION OBJECTIVE.

The oil-immersion objective, or homogeneous system, is so constructed that when in use the pencil of light passing through the object to the objective traverses only media of the same refractive index. This is accomplished by placing between the cover-glass and the end of the objective a medium having the same refractive index as glass. To accomplish this a drop of cedar oil is placed upon the cover-glass, and the objective brought into contact with this, and the observation made through the oil. This class of lens is intended to work only with the oil, and is unsatisfactory when used dry. Frequently it is not convenient to use cedar oil for the preliminary examination often employed to determine the progress of staining, etc.; here the staining fluid may be washed off and a drop of water used in place of the oil; this will give a sufficiently good picture for the purpose, and has the advantage of not interfering with the addition of further stain when desired.

The tube or barrel of the microscope is made in two forms, the long or English type being from 8 to 10 inches, and the short or Continental type having a length of 6½ inches. The latter is the more desirable form, since the majority of manufacturers of objectives adjust them for the short tube.

APOCHROMATIC OBJECTIVES.

This term is applied to a particular variety of lens containing a special kind of glass (containing calcium fluoride), besides the usual crown and flint glass, the object of this being to produce a greater degree of achromatism, thus reducing chromatic aberration. The special value of these objectives when used with a compensating ocular (compensating eye-pieces are specially constructed for use with apochromatic objectives), is as follows: Three rays of the colored spectrum, instead of two, as in case of the achromatic glasses, are focused in the same plane, leaving only a minute tertiary spectrum; also with these objectives the spherical aberration is corrected for two

colors in the brightest part of the spectrum, and the objective shows the same correction for marginal rays as for the central part of the aperture. Finally, apochromatic objectives admit the use of highly magnifying oculars. This form of objective is particularly useful in photomicrographic work, where it is very important to abolish chromatic aberration.

ILLUMINATION.

Illumination constitutes a most important factor in the practical use of the microscope, and upon its proper management depends very much of the efficiency of the work. Direct, unmodified sunlight is unsuited, and should not be employed, for general work. North light is the most uniform and steady, and is to be preferred. The use of artificial light should, as far as possible, be avoided, and when it must be used its character and color may be greatly improved by inserting a piece of blue glass between the reflector and the object. It is always necessary to keep the eye close to the ocular and if possible to keep the unemployed eye open. Too much light is always to be avoided, as it is only an added strain to the eyes, accomplishes nothing, and may materially interfere with the efficiency of the work.

Oblique Light.—For urinary work the oblique light is best. By this term is meant light in which the parallel rays from the plane mirror meet the optical axis of the microscope at an angle. This form of light may be obtained in the following ways: (*a*) By placing the reflector to one side of the stage; this results in an oblique ray without materially lessening the strength of the light; the condenser must, of course, be swung out of the way. In microscopes which have a fixed mirror, *i.e.*, only movable perpendicularly at right angles to the axis, oblique light may be obtained through the condenser, as follows: (*b*) First, focus the light upon the object through the condenser; then, lower the condenser until its focus is considerably below the plane of the object; thus the rays emerging from the condenser will decussate and then diverge, so that all but axial rays will fall upon the object in an oblique direction.

DARK-GROUND ILLUMINATION.

The Substage Dark-ground Illuminator.—Most high-grade microscopes are now fitted with a substage dark-ground condenser. (See Fig. 2.) If this is available, the microscope should be set in a vertical position and the dark-ground condenser substituted for the regular Abbe. If daylight is used, no paralleling device is necessary. If artificial light is used a bulls-eye condenser must be interposed between the substage reflector and the light, in such a way that parallel rays strike and completely cover the plain substage mirror. Then focus the two concentric



FIG. 2.—DARK-FIELD ILLUMINATOR.

rings engraved upon the upper surface of the condenser and center them accurately by means of the screws provided for this purpose.

Preparation of Fresh Specimen.—When employing the dark-field condenser the material to be examined should be in a fresh, moist state, if the best results are to be obtained. Carefully smear the specimen to be examined upon one or more slides, preferably new ones that have not been scratched by handling, and cover with thoroughly cleansed new No. 1 cover-glasses. Place a large drop of immersion-oil upon the upper surface of the condenser and then fasten the specimen on the microscope stage, bringing the condenser up until the immersion-oil is in contact with the under surface of the slide. A properly adjusted mirror should show a bright spot in the center of the mounted slide. Place a drop of cedar oil in the center of the cover-glass and bring the intermediate objective into contact with the drop

of immersion-oil; focus the bright spot already referred to, and if it does not occupy the center of the field adjust the substage mirror until it does. If these steps are properly carried out, and the object to be examined is properly in focus, then the intensely illuminated bacteria, spirochæte, etc., will stand out sharply against a dark or black background.

IMPROVISED DARK-FIELD ILLUMINATOR.

For one not possessing a dark-field illuminator, a very satisfactory substitute can be made by having a piece of thin metal



FIG. 3.—FORM AND SIZE OF METALLIC STOP, FURNISHED WITH THE LEITZ MICROSCOPE, USED IN CONJUNCTION WITH THE ABBE CONDENSER, TO PRODUCE DARK-FIELD ILLUMINATION.

cut in the form shown in the illustration (Fig. 2) of such a size that the outer narrow ring fits snugly into the movable ring provided in most substage Abbe condensers. This special stop is now furnished with most microscopes. A little experience will indicate the proper size of the central disc, which when properly adjusted should produce conditions similar to those described in the preceding paragraph. The relation of condenser, specimen, and objective is the same as with the regular dark-field illuminator. Results with this apparatus, while not perfect, are very satisfactory and may be relied upon for demonstrating spirochæte and other micro-organisms. It has the advantage of allowing the examiner to rapidly shift from the dark to the light field without disturbing the relation of the objective, specimen or condenser.

TO CLEAN THE MICROSCOPE.

Should the lenses or oculars become blurred or spotted from dust or dirt, proceed as follows: If the dirt is upon the ocular it will be discovered by rotating the ocular within the barrel while observing the illuminated field of the microscope. If the obstruction is upon the ocular it will be seen to move; if upon the objective it will remain stationary during this manipulation. To find an obstruction upon the objective rotate it, when the spot or mark will move with it. Finally, if after testing both the objective and ocular in the manner described above the location of the dirt or dust is not demonstrated, the trouble will then be found upon the glasses or spectacles if the observer happens to wear them. Having located the trouble, remove the affected part and cleanse as follows: If careful polishing with bibulous paper or fat-free silk fails to accomplish the desired result, then moisten the silk or paper with a trace of distilled water, using this to aid in the removal, finally polishing dry. For oily or resinous smears a small amount of alcohol may be used. Special care should then be exercised to avoid any excess of the solvent which may enter between the individual lenses and, dissolving the cement, ruin the part. After using the oil-immersion it should always be cleaned before leaving the instrument. After removing the excess of oil with the silk, wipe off the remainder with a trace of xylol or benzine, immediately wiping dry. Cover-glass preparations may be treated in a similar way.

Glass surfaces should never be touched with the fingers, and great care should be exercised to avoid dropping and possible fracture of the oculars and objective. All *metal* parts of the instrument should be kept free from liquids, particularly acids and alkalies, benzine, xylol, alcohol, turpentine, and chloroform.

To clean the mechanical parts, none but the best machine oil should be used, and this only in small amounts and at long intervals. The polished brass requires nothing but occasionally polishing with clean chamois.

Two keynotes to successful use and preservation of the microscope are: handle with care and keep scrupulously clean.

THE MECHANICAL STAGE.

Of the many aids to exact work in the realm of clinical medicine the mechanical stage is of great practicability and wide application. Every possessor of a microscope should aspire to the possession of a mechanical stage, for this mechanism is not only a great time saver, but will materially aid in the search for bacteria and pathologic cells in the blood, and is practically a necessity in making a differential count.

Description.—Referring to Fig. 4, the general appearance of this instrument will be seen. It is designed for application to the stage of the microscope, upon which it is rigidly fastened

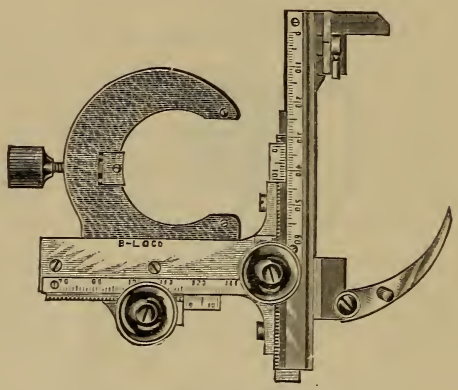


FIG. 4.—MECHANICAL STAGE.

by a collar and set screw attached to the stand. It is important in this connection to note that most manufacturers of microscopes make a mechanical stage for their particular instrument, and which frequently is not interchangeable with microscopes of other manufacture, so that, before purchasing, one should be certain that the stage will fit the instrument for which it is intended. When attached to the stand the slide carrier should move in both directions without any undue force and without any irregularity or jarring of the mechanism, and to the limits of its movement should remain in close and even contact with the microscope stage.

The mechanical stage is fitted with two milled screws which are movable in either direction, and which convey gradual

motion to the slide held in the jaws of the instrument. One of these screws controls the vertical, and the other the horizontal, motion, so that by proper manipulation it is possible to rapidly and accurately go over the whole of a specimen. (For more detailed description of the use of the mechanical stage in the differential count, see chapter on "The Blood.")

Most mechanical stages are provided with a millimeter scale and vernier reading to 0.1 of a millimeter, which serves not only to measure the size of small objects upon the stage, but is also very useful for locating objects of special interest upon any slide, so that they may again be found without difficulty. (See below the Maltwood finder and Pepper's application without the mechanical stage.)

THE WARM STAGE.

A further differentiation and improvement upon the mechanical stage is the stage prepared for preserving specimens at body-temperature during examination. This is provided with a thermometer which indicates the temperature of the object during examination. An extemporaneous warm stage has been described in another section (Parasites) to which the reader is referred.

THE MALTWOOD FINDER.

In microscopic work, especially in studying blood or bacteriologic slides, some sort of "finder" is an essential part of the equipment. While the vernier scale, attached to most mechanical stages, is fairly satisfactory for individual work, it does not fulfill all conditions demanded of it, since the same stage and microscope must always be used, and if by accident the relation between microscope and stage is altered ever so little, then all previous figures indicating location are rendered valueless.

The Maltwood finder (Fig. 5) does not possess the above disadvantages, and can be used universally with uniform results.

The Maltwood finder[1] consists of a heavy glass slide comparing exactly in size with the ordinary microscope slide. The central third of this slide is covered with a close network of

[1] Wm. Pepper: Jour. Amer. Med. Assoc., July 20, 1908.

intersecting rectilinear lines which form a large number of uniform squares. Each square contains two figures arranged one above the other, so that no two squares represent the same combination (see below). This marking has been placed upon the slide by a photographic process.

1 1	1 2	1 3	1 4	1 5
2 1	2 2	2 3	2 4	2 5
3 1	3 2	3 3	3 4	3 5

All Maltwood finders are made interchangeable, the squares coinciding exactly in all slides.

Method of Using the Finder.—If on looking over the slide with the mechanical stage a part of the field is discovered which

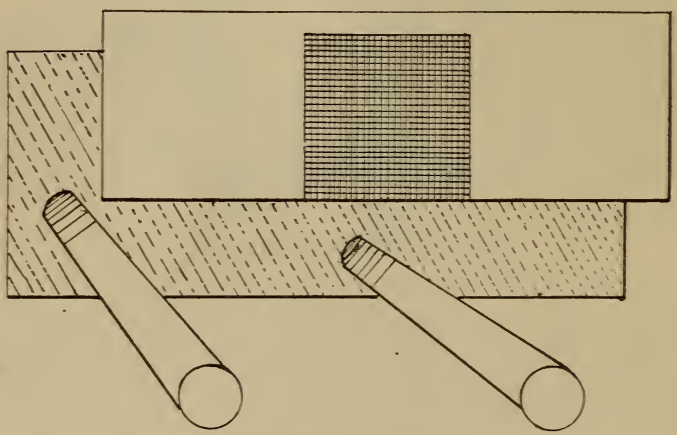


FIG. 5.—MALTWOOD FINDER, SHOWING DR. PEPPER'S BRASS ANGLE SUBSTITUTE FOR MECHANICAL STAGE.

one desires to examine at a later time, the slide is carefully removed without altering the location of the mechanical stage and the Maltwood finder substituted; the square is then observed which corresponds with the center of the slide. This combination is to be jotted down, and then in order to make sure that the proper reading has been made, the finder and slide should be substituted for each other a number of times. Sup-

pose this reading was made $\left[\frac{19}{21}\right]$, then, when later this particular field is sought the above process is reversed, and when the recorded square is in the center of the field, the slide is carefully substituted, when the object sought will appear in the field.

One of the chief advantages of the finder is that the slide may be sent to any one having a finder, with a note to look for such or such a square. For those not possessing a mechanical stage, Dr. W. Pepper has devised a small right angle of brass which may be easily carried in the pocket, and which may at any time be substituted for the mechanical stage, as the only purpose of the mechanical stage is to afford a fixed angle into which the slide and finder may be fitted.

The illustration shows the arrangement of slide and brass angle, and illustrates the size of the right angle compared to the slide. The strip of brass from which the form is cut should be about one-sixteenth inch thick.

EXAMINATION OF URINARY SEDIMENTS.

Preparation of the Slide.—After concentration of the sediment by centrifugation or sedimentation, the next point is to arrange this collection upon the slide for examination under the microscope. For this examination a few drops may be taken up in a pipette and allowed to fall upon the center of a clean slide, upon which a clean cover-glass is immediately placed. This will exclude dust, prevent rapid drying during examination, and will so flatten the field that frequent change in focus will not be necessary, while the different elements are more easily viewed and differentiated owing to their uniform separation and lack of overlapping.

The proper removal of the sediment from the bottom of the container is an all-important part of this process, and upon this the successful finding of casts will frequently depend. This is best accomplished with a small nipple-pipette or section of narrow glass-tubing, one end of which is drawn out into a coarse capillary point. Having cleansed the slide and cover slip, arrange them in a convenient place and then proceed to remove a few drops of sediment from the bottom of the tube. It is absolutely necessary, during this procedure, to prevent the entrance of an excess of fluid into the pipette. This end is accom-

plished in the case of the pipette by carrying it down into the fluid nearly to the sediment, then expressing a few bubbles of air before passing the tip beneath the sediment. With the straight tube the moist thumb or finger is held firmly upon the upper opening of the tube while its tip is carried to the bottom of the vessel, then by means of a slight rotation of the tube while relaxing the pressure above, a few drops of sediment are allowed to enter the tube. Firm closure of the upper end of the pipette is maintained while the tube is withdrawn and its contents transferred to the slide. To further guard against diluting the sediment, the outside of the tube should be wiped dry after it has been removed from the urine.

Special Technic for Excessive Sediments of Phosphates, Pus, etc.—It is not uncommon to meet with urinary sediments of such volume and density that the grosser condition may completely obscure the more important but less numerous elements, such as casts, pus, blood-cells, etc. In such cases the following technic is advised: After taking up the sediment deposit but one drop upon the slide; then from a clean pipette add a few drops of distilled water or, what is better, the clear urine from above the sediment. Agitate this till evenly mixed, and then apply the cover-glass and remove excess of fluid with strips of filter- or blotting-paper. Thus diluted not only is there better opportunity of finding casts, but the internal structure of the different elements will be much clearer.

Microscopic Search.—After preparation of the specimen as above outlined, the slide is transferred to the horizontal stage and first viewed by low power and a rather subdued light. In this examination it is safer to begin with the objective below the focal point, then with the eye in position gradually rack upward, at the same time keeping the slide moving with the other hand. If this is continued carefully there will be little difficulty in discovering the objects in the sediment, even if few and small. Another advantage of this plan is that all danger of crushing the slide or fracturing the objective by forcibly racking down is eliminated, since the objective is never carried downward except when the relation of objective to slide is plainly seen.

Having focused the sediment and regulated the light, the

slide should be searched as follows: Slowly move the slide forward until one corner of the cover-glass appears in the field, then locate the lower right-hand corner and gradually move the slide upward in as straight a line as possible, until the opposite border is reached. Then move slightly to the left and proceed in a straight line downward to the lower margin again. This procedure is repeated until the whole area of the cover-glass has been searched. For those having a mechanical stage the slide may be placed in this, and the same movements carried out with its aid. In either case it is well to keep one hand upon the fine adjustment to sharpen up any obscure objects which may be encountered.

EXAMINATION OF THE BLOOD.

For an examination of fresh blood the 6 objective is most suited, since nothing but the cell outlines can be seen with the 3. A good light, with the condenser in close apposition with the under surface of the slide, will give the best results. To differentiate the varieties of white cells by their nuclei and granulation requires very careful regulation of the light, since the very slight difference in refraction of nuclei and protoplasm makes the differentiation extremely difficult at best.

Counting the Corpuscles: Red Cells.—Having made the proper dilution and mounted the specimen as outlined in the section on blood, the slide is placed upon the stage and a few minutes allowed to pass while the corpuscles settle to the bottom of the chamber. The medium power, 6, is brought into close apposition with the cover-glass, and then with the eye to the ocular the fine adjustment will bring the individual cells into view. Considerable difficulty is at times experienced in locating the part of the counting chamber containing the ruled lines. This may be overcome by centering the inner circle of the chamber by means of the outside of the objective. If this is carefully done the first attempt at focusing will usually bring the squares into view. Some hemocytometer slides are very faintly ruled, rendering the operation of counting very difficult. This may, in a measure, be overcome by reducing the light, at the same time taking advantage of the slight shadow caused by oblique rays coming from the lowered condenser. If this is done

great care should be observed in determining border-line cells on account of the uncertain shadow cast by all cells with this illumination.

The White Cells.—Either the 3 or the 6 objective may be used in this connection, and of these the 3 is generally preferred. It does, however, require a little more care and experience to make an accurate count, because of the minute appearance of the individual cells. Its chief advantage is that it is not necessary to move the slide while counting the entire field, after it has once been centered, thus doing away with the error of missed squares which occasionally occurs in moving the

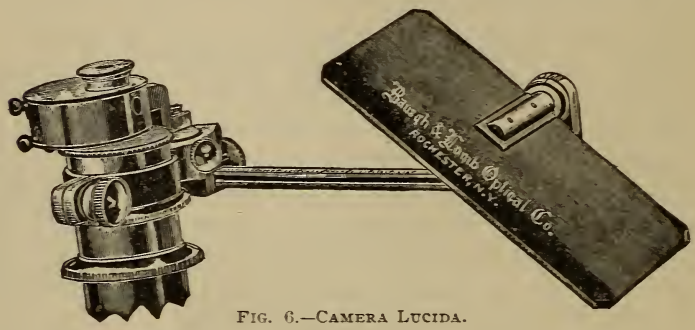


FIG. 6.—CAMERA LUCIDA.

slide when using the 6 objective. Here, again, low illumination and oblique rays may advantageously be employed.

Examination of the Stained Specimen.—Since the usual reason for staining and examining the dried specimen is to study minute structural characteristics, to make a differential count or to discover the presence of bacteria or parasites, it is necessary to employ the oil-immersion lens and to occasionally supplement this with a highly magnifying ocular. In this work good illumination and sharp definition (condenser close to slide) are essential.

THE CAMERA LUCIDA

The CAMERA LUCIDA is a device by the aid of which it is possible to quickly and accurately trace upon paper the magnified image of any microscopic object upon the microscope stage. It will be found of particular value in parasitology, where it is

required to study in detail the minute anatomy of organisms too large to be viewed at one time under the microscope.

This apparatus is essentially a combination of mirrors and lenses by which the image on the sheet of paper is reduced by a suitable lens and is projected into the field of the microscope, so that the eye of the observer at the eye-piece of the camera lucida sees the image of the object and the paper and pencil of the examiner at the same time, *i.e.*, apparently the enlarged object has been transferred to the sheet of paper, so that the pencil, as followed by the eye, can be made to trace the lines of the object upon the paper.

The essential parts of the apparatus are the eye-piece, which is arranged to clamp firmly above the ocular of the microscope in the optical axis of the instrument. This contains mirrors and lenses suitably arranged to reduce and deflect the image projected from the large reflecting mirror situated to the right of the ocular. This large mirror serves to reflect the image of the paper and pencil tip into the condensing lens of the camera lucida.

In setting up this instrument, the circular clamp on the camera lucida is firmly attached to the upper part of the ocular, and the horizontal bar carrying the reflecting mirror is clamped into the guide provided for that purpose. The camera lucida is so hinged to the clamp that it can be swung out of the way while locating the proper field in the microscope, after which it can be swung into place and centered by means of two small setscrews. The reflecting mirror is also arranged so that its angle can be adjusted between 45 and 80 degrees.

THE MICROMETER.

This is an instrument used for measuring the size of bacteria or other objects, when viewed through a microscope.

The unit of length employed in micrometry is the one-thousandth part of a millimeter (0.001 mm.), called for short a micron and indicated by the Greek letter P.

The first requirement is a stage micrometer; this is a slide having engraved on it a scale divided into hundredths of a millimeter (0.01 mm.), every tenth line being longer than the others

to facilitate counting. The face of this scale is protected by a cover-glass, which is cemented over it.

The measuring is done with a camera lucida in conjunction with the stage micrometer as follows:—

Adjust the camera lucida to the eye-piece of the microscope, then adjust the micrometer on the stage of the microscope and accurately focus the division. Project the scale upon a piece of paper and with a pen draw accurately the magnified image of the scale. This is preserved for future use. In using this paper scale, the same combination of ocular, objective, and tube-length should always be maintained. A similar scale to suit all combinations may be made in a like manner.

Eye-piece Micrometer.—This is a circular glass disc having engraved on it a scale divided into tenths of a millimeter (0.1 mm.). This is fitted inside the ocular in such a way that it rests upon the central diaphragm. When in this position it is in most instruments exactly in focus of the eye-lens.

This eye-piece micrometer must then have the value of its spaces, according to each optical combination, estimated with the stage micrometer and the measure of the spaces together with the optical combination should be recorded for future use.

In measuring an object by this method, read off the number of divisions of the eye-piece micrometer which it covers, and express the result in microns by reference to the corrected table previously prepared.

II.

THE SPUTUM.

GENERAL CONSIDERATIONS.

Sputum, or expectoration, is the product of inflammatory reaction of the bronchial and lung tissue and is voided by coughing or clearing the throat. It is composed of the secretion and the exudate from the mucous membrane of the nose, pharynx, and trachea, down to the finest bronchioles and alveoli; also material that may have entered the respiratory tract from adjacent organs (pus of abscesses and empyema); blood derived from any part of the respiratory tract; and of material coming from the buccal cavity and from any part of the digestive tract.

On account of this complex origin the composition of the sputum is very variable. The sputum may be present, but may not be expectorated. Small children and occasionally adults, on account of bad habits, insufficient practice, or impaired consciousness, swallow their sputum. In the majority of cases this difficulty can be remedied by training. For diagnostic purposes the total output of sputum for twenty-four hours is collected in a suitable receptacle, and should be free from admixture of antiseptics.

The sputum is examined in bulk with reference to its quantity, reaction, consistence, air-content, apparent composition, color, and odor.

PHYSICAL AND CHEMICAL CHARACTERISTICS.

The amount of sputum voided in twenty-four hours may be very great. On the other hand, even when every effort is made to expectorate, very little is produced. Some phthisical patients, in spite of violent coughing, raise very little, and that is of a very tenacious quality.

Scanty, or *absent,* sputum may be evidence of the first stage of bronchitis, asthma, laryngitis, or pleurisy, while in children

under 6 or 7 years of age it is usually absent, because it is swallowed.

Abundant sputum is of importance in a general way because it denotes, in acute infectious conditions particularly, that nature is prompt to relieve the body of an abundant secretion, which, if retained, might cause serious consequences by further reducing the already diminished respiratory capacity. When large amounts of sputum are voided at short intervals, alternated with periods of practical absence, one may infer a tuberculous, gangrenous, or bronchiectatic activity, or rupture into the lung of an abscess of the lung, liver, kidney, or subphrenic space.

Macroscopic Appearance.—The gross appearance of sputum, apart from its general consistency, is largely dependent upon the inclusion of adventitious material, and is clinically described under the following heads:—

The *consistence* of sputum may bear a certain relation to the amount when, if abundant, the consistence is lessened, and *vice versâ*. This relation is by no means constant.

Ordinarily, sputum is "slimy." It may, however, be serous, purulent, or bloody. The peculiar slimy characteristic of sputum depends upon the amount of mucus contained, while its stickiness depends, in part, upon the mucin and, in part, upon the proteid content. This is especially marked in lobar pneumonia.

(a) *Watery, or serous, sputum,* which is frequently blood-tinged, occurs in pulmonary edema, and in catarrhal influenza. Gastric disorders in neurotic old people may give rise to a thin, watery expectoration of considerable quantity, which is partly regurgitated and partly hawked up.

(b) *Viscid, or sticky, sputum,* which adheres to the bottom of the container, even when completely inverted, is somewhat characteristic of lobar pneumonia, but may also be seen in phthisis, pertussis, and in broncho-pneumonia.

(c) *Mucoid sputum,* is a clear, diffluent sputum resembling egg-albumin, composed chiefly of mucus, is observed in the early stage of pneumonia, bronchitis, and phthisis; at the termination of an asthmatic attack; in pertussis, pharyngitis, laryngitis, measles, and influenza.

(d) *Muco-purulent sputum* is composed of mucoid sputum in which occurs a varying number of streaks and masses of opaque, yellow or greenish pus. It is noted toward the end of measles, in pertussis, in resolving pneumonia, during phthisis, and in subacute and chronic bronchitis.

(e) *Purulent sputum,* which is composed purely of pus, is rather rare, and, when observed, indicates rupture of a liver, kidney or subphrenic abscess, or a purulent pleurisy into the respiratory tract. Opaque yellow sputum, consisting largely of pus, is found in bronchiectasis, phthisical cavities, broncho-pneumonia, and in the chronic or later stages of acute bronchitis.

(f) *Nummular sputum.* Ring or coin-shaped masses of sputum, which sink immediately in water, occur at times in bronchiectasis, chronic bronchitis or phthisical cavity.

(g) *Frothy sputum* may be observed in bronchitis, broncho-pneumonia, and emphysema. Its most important relation is in pulmonary edema, in which condition it is full of air and resembles frothy soap-water.

Color.—(a) *Rusty sputum,* due to evenly distributed altered blood, is generally indicative of lobar pneumonia, but may also be observed in tuberculosis pulmonalis.

(b) *Prune-juice expectoration,* is a rather fluid expectoration discolored by altered blood, and is seen in gangrene and in cancer of the lung.

(c) *Currant-jelly sputum* is said to be characteristic of cancer of the lung.

(d) *Black sputum.* Sputum which is very dark or black streaked or specked is found in persons who have inhaled coal dust or smoke for long periods of time. It is sometimes seen in gangrene of the lung.

(e) *Yellow, or green sputum* may be caused by abscess of the liver which has ruptured into a bronchus (bile pigment), and is also seen in some cases of pneumonia (altered blood), and in pulmonary infections with chromogenic bacteria.

(f) *Shreds and casts* may be observed in the sputum of chronic bronchitis, diphtheria, and rarely in pneumonia. Casts, unless large and branching (Fig. 7), are more apt to be found during microscopic search. Suspicious particles should be

floated in water against a black background, and teased out with needles.

(g) *Blood-streaked sputum.* Sputum streaked or discolored with blood may be due to violent vomiting or coughing, diseased tonsils or leakage from an aortic aneurism. Under these conditions it will appear as light red, but slightly altered



FIG. 7.—BRONCHIAL CAST, FROM CASE OF FIBRINOUS BRONCHITIS IN SERVICE OF DR. JUDSON DALAND (ORIGINAL).

blood. It may be present as a sequel of hemoptysis or abscess of the lung in broncho-pneumonia, empyema or in bronchitis.

If the blood is dark or black it may be due to pulmonary infarction. Most commonly hemoptysis is observed in phthisis, when it recurs intermittently for days or weeks. Finally, it should be remembered that malingerers may simulate disease of the respiratory tract by sucking blood from wounds of the gums, lips, tongue, or cheeks.

(h) *Hemorrhagic sputum* is observed in traumatic or tuberculous hemorrhage, in hemorrhagic infarctions, and in lobar

pneumonia. Also in tumors in or near the respiratory tract, and finally in congestion of the pulmonary circulation.

Certain derivatives from the blood-pigment produce in the sputum shades very similar to that of blood—the *rusty sputum* of pneumonia, for instance. The coloring matter here is partly changed blood and partly a yellowish-red derivative of blood-pigment, about which little is known. Peculiar lemon-colored and grass-green shades are also frequently observed in pneumonic sputum. These likewise are due to changed blood-pigment. Such sputa respond to Gmelin's test for bile-pigment (see page 283). A peculiar light-brown sputum is sometimes observed in heart disease, particularly mitral, in which amorphous blood-pigment is found encapsulated in the alveolar epithelium (heart-failure cells). A type of green sputum has been observed in cases of lung tumor; the nature of the pigment in question is as yet unknown.

(i) *Extraneous discolorations.* Other noticeable discolorations of the sputum are observed from the admixture of inhaled dust-particles. The *black sputum* of miners, and the *blue sputum* of workers in ultramarine, are examples of this class.

Finally, it should be remembered that the sputum voided, following the ingestion of certain food-stuffs, may be discolored from contamination with these occurring in the mouth and pharynx. A *greenish* discoloration of the sputum is sometimes the result of the activity of certain chromogenic bacteria, especially the *Bacillus virescens.*[1] *Yellow* and *bluish* sputa of probable bacterial origin have occasionally been observed.

The *reaction* of fresh sputum is generally alkaline; it may occasionally be acid, and usually becomes so after standing for some time through decomposition resulting from bacterial growth.

Air Content.—Sputum is often more or less foamy or frothy, due to the presence of air. Other things being equal, the content of air in the sputum is greater the finer the bronchi from which the sputum is derived. The consistence of sputum often has a bearing on this. The amount of air contained can easily be determined by comparing its specific gravity to that of

[1] Frick: Virchow's Archiv, vol. cxvi, 1889.

water. Air-containing sputum will float; airless sputum will sink.

The odor of fresh sputum is rather characteristic, but indescribable. On standing it may acquire a disagreeably nauseating odor from decomposition, resulting from contained bacteria. Freshly voided sputum has a very decided odor in bronchiectasis, purulent bronchitis, tuberculosis, gangrene of the lung, and lung abscess. The disagreeable odor here arises from the activity of putrefactive bacteria. Stagnation of the secretion in cavities favors decomposition. In this way the foul odor of the expectoration in consumptives may be imparted to the breath. Finally, it must be remembered that fecal vomiting and diseased conditions of the mouth may be responsible for the odor of the breath and sputum, as will also the ingestion of certain volatile drugs, including alcohol.

HEMOPTYSIS.

True hemoptysis means the expectoration of an appreciable amount of pure, or nearly pure, blood, not merely sputum tinged with blood. The amount of blood may continue small, and may persist for many days or it may, as in the case of rupture of an aortic aneurism, be sufficiently large to cause death in a short time.

Before making a diagnosis of hemoptysis it is necessary to exclude blood coming from the nose, pharynx, larynx, buccal cavity, and tonsils.

The Common Causes of Hemoptysis.—(a) *Pulmonary disease,* usually tuberculous. It may also occur in the early stages of lobar pneumonia, abscess, bronchiectasis, gangrene, and cancer of the lung.

(b) *Cardiac disease.* Here it may be the result of venous obstruction occurring in the course of valvular disease. This is a not uncommon cause of slight but long-continued bleeding.

(c) *Vascular disease.* The most important condition is rupture of an aneurism into the respiratory tract. Leakage from the same cause may cause slight but persistent hemoptysis.

(d) *Diseases of the blood.* Hemoptysis may occur during the course of hemophilia, purpura, leukemia, scurvy, and severe

anemia. It has been noted occasionally in the course of some of the exanthemata.

(e) *Miscellaneous causes.* Vicarious menstruation and hysteria.

MACROSCOPIC EXAMINATION OF SPUTUM.

Examination should be made both upon a white and upon a black background. Many sputa appear to the naked eye to be homogeneous—pure mucus, pure pus, pure blood; but sometimes not only may the sputum of one patient vary, but differences may be noted in each expectoration.

"Dittrich's plugs" are yellowish-white masses, the size of a mustard-seed, and are easily seen over a black background. They come from the smaller bronchioles in putrid diseases, especially in putrid bronchitis and pulmonary gangrene. Microscopically they are composed of clumps of bacteria and fatty acid crystals. They have an intense and very disagreeable odor. Somewhat similar plugs may be encountered in the sputum in follicular tonsillitis. These plugs should not be confounded with those little masses described by, and known as, Curschmann's spirals (see page 27), or with the characteristic cheesy masses seen in the sputum of pulmonary tuberculosis.

Formations consisting of *fibrin* are encountered in certain infections, and should be easily recognized by their white color, tenacious consistence, and sometimes by their shape (casts and molds).

Foreign bodies are sometimes aspirated into the respiratory tract, where they may remain for years without causing any symptoms until a fit of coughing dislodges them and they appear in the sputum.

Concretions, known as broncholiths, or pneumoliths, depending on their origin, are sometimes, though very rarely, formed in the lungs during chronic inflammatory conditions. These are accidentally coughed up, when they may be found in the sputum. Occasionally these stones may have their origin in the crypts of the tonsils, or they may be calcified lymph-nodes that have ulcerated into the lung.

MICROSCOPIC EXAMINATION OF SPUTUM.

A microscopic examination of the sputum reveals the presence of cells, elastic fibers, casts, spirals, crystals, and microorganisms. It is advisable to examine first the fresh unstained sputum, as the presence of fungi or crystals in the sputum, and the nature of many of the cellular elements can be determined only in this way. Afterward dried and stained cover-glass preparations may be made for more minute and detailed study.

Preliminary Examination.—This is necessary in order to locate suspicious particles which may be scattered throughout the large mass of sputum. This is made either with the unaided eye or with a hand-lens. A thin layer of sputum is necessary to successful examination. For this purpose a moderate-sized Petri dish and cover is much better than the flat pieces of glass ordinarily employed, which are uncleanly and difficult to handle. A small amount of sputum is placed on the inside of the cover, and the other half of the dish pressed down into this, the rim very successfully preventing the escape of excess of material.

The Unstained Specimen.—For the purpose of isolating any characteristic particles, the sputum should be spread out in a thin layer in the dish, and the material teased out with needles or tooth-picks. Having located a likely particle, it is transferred to a clean slide and flattened out by pressing a cover-glass down upon it. This should be examined first by the low, and later by the medium, power objective.

Appearance.—Most sputum consists, microscopically, of a ground-work or *mucous matrix* of indefinite structure and appearance, in which are imbedded a variety of microscopic objects, principally cells:—

1. *Pus cells.* The number of these indicates, in a general way, the purulent nature of the specimen. The character of the corpuscles varies greatly. Their size is from 7 to 10 micromillimeters, they appear to be more or less granular, are sometimes distinctly pigmented, containing one or more irregular nuclei. The granules are composed, some of proteid, some of fat, and some of extraneous *débris.*

2. *Epithelial cells* found in the sputum differ from the pus cells, being usually larger in size, and by exhibiting one rather

large vesicular nucleus. Various types of epithelia are met in the sputum, and their recognition is of considerable value in locating the origin of the expectoration, although, many times, the conditions to which they have been subjected after being shed have so altered their appearance that little information can be gained from their study: (*a*) Squamous epithelia are derived from the mouth, the pharynx, and from part of the larynx. (*b*) The cylindrical epithelia are derived from the nose or from the smaller bronchi, and are seen as pear-shaped or oval cells, some of which possess cilia. (*c*) Pulmonary, or alveolar epithelia are oval and measure from 20 to 30 micromillimeters in diameter.

3. *"Heart-failure" cells.* These are either oval or round, pigmented alveolar cells. When numerous their presence is said to be indicative of chronic passive congestion of the lungs, usually depending on the failing compensation of cardiac valvular disease. Their presence is, therefore, usually associated with the common signs in the lungs, which are indicative of failing compensation, viz.: moist râles, mucous expectoration, and cyanosis.

4. *Eosinophiles* may occasionally be found in large numbers associated with Charcot-Leyden crystals in the expectoration of bronchial asthma.

5. *Red blood-cells.* The appearance of red blood-cells in the sputum will depend largely upon the length of time that they have been shed. As they grow old they become pale, shadowy, and fragmented. The finding of a few red blood-cells in the sputum is of no diagnostic import. They occur naturally in large numbers in hemoptysis, and are constant and more or less abundant in all inflammatory diseases of the lungs, particularly phthisis.

6. *Casts.* These may vary in size from those which represent molds of the trachea and larger bronchi (see Fig. 7) to those coming from the smaller bronchioles, and which are from ¼ to 1½ inches long. These smaller casts are the more common, and when present usually require the use of the low-power objective to demonstrate them. These casts usually occur in the three following diseases: the largest in diphtheria, medium size in fibrinous bronchitis, and the smallest in lobar pneumonia.

7. *Curschmann's spirals* consist of worm-like spirals 1 to 2 centimeters long, and about 1 millimeter wide. They are

more or less opaque, and are usually found surrounded by a thick, clear mass of mucus. They frequently show a central, undulating, thread-like core around which are twisted, in a spiral manner, the mucous threads. Entangled in these spirals are usually eosinophiles and Charcot-Leyden crystals. The spirals occur frequently in the sputum of bronchial asthma, more rarely in phthisis, bronchitis, and in lobar pneumonia. Their presence may be of service in differentiating bronchial from other forms of asthma.

Crystals are never found in the freshly formed sputum, but are indicative of stagnation of the material within the body or of decomposition after being expectorated. The crystals usually met in the sputum are: (a) *Crystals of fat or of fatty acids* appear as long, slender needles, either singly or grouped into fine rosettes or sheaves. They are readily soluble in potassium hydrate or in ether. This solubility is easily determined by allowing a little of either fluid to flow under the edge of the cover-glass while observing the crystals in question.

(b) *Crystals of calcium phosphate* may be encountered under conditions of retention and stagnation.

(c) *Charcot-Leyden crystals* are occasionally encountered, particularly in the expectoration of bronchial asthma, and are here accompanied by eosinophiles. They appear as colorless, elongated double pyramids, varying considerably in size. They are often so small that high magnification is required to reveal them.

(d) *Cholesterin crystals* are but rarely seen in the sputum. They occur as transparent, colorless, rhomboidal platelets, with notched or irregular angles and ends.

(e) *Hematoïdin crystals* are derived from hemoglobin by a process of decomposition, and occur as needles and rhomboidal platelets of reddish and brownish hue. They are found chiefly in the sputum from old abscesses or perforating empyemas.

(f) *Leucin globules and tryosin crystals* are found in putrid sputum from old perforating abscesses or in putrid bronchitis.

(g) *Calcium oxalate,* in minute octahedral crystals, are occasionally met.

Elastic Fibers.—When the lung-tissue is destroyed to

any extent by pathologic processes, elastic fibers are likely to be encountered in the sputum. Their presence in the sputum proves conclusively the existence of some destructive process within the lung. Hence their importance in the diagnosis of tuberculosis of the lung before the appearance of tubercule bacilli. As the healing process begins and progresses this elastic tissue gradually diminishes in amount, so that a constant presence or an increase in the amount indicates a progressive condition. Elastic tissue is also seen in abscesses of the lung, in bronchiectasis, in pulmonary infarct, occasionally in pneumonia, and in cases of gangrene of the lungs.

These fibers may usually be detected in a thin layer of sputum examined microscopically with the medium or low power. Care must be observed to avoid confusing true elastic fibers with the somewhat similar vegetable fibers, which latter are generally larger and less uniformly wavy.

Methods of Separating Elastic Tissue for Examination.— To detect particles or shreds of elastic tissue in the sputum, suspicious lumps should be thoroughly mixed with an equal quantity of 20 per cent. sodium hydrate solution; then a large volume of water added, and the whole allowed to sediment for a few hours. The sediment is then removed and examined under the microscope for the characteristic fibrillated masses.

If the elastic tissue is not found by this procedure, the entire quantity of the twenty-four-hour specimen should be boiled with an equal quantity of 20 per cent. sodium hydrate solution. The resulting gelatinous mass is then mixed with several volumes of water, and allowed to sediment, the supernatant fluid poured off and about 15 cubic centimeters of the sediment centrifuged for fifteen minutes. The final precipitate is now carefully removed and examined as above.

STAIN FOR ELASTIC TISSUE IN THE SPUTUM.

Elastic tissue may be demonstrated by the orcein stain of Unna-Tanzar (see appendix for stain). In using this stain the suspected material is treated with a few cubic centimeters of the dye on a slide and then warmed for five minutes, after which the preparation is decolorized with acid alcohol. The elastic tissue fibers will be stained a brownish violet by this process.

RARER DIAGNOSIS MADE BY EXAMINATION OF THE SPUTUM.

Occasionally evidence of disease adjacent to the respiratory tract may be obtained by an examination of the sputum. Thus fragments of tumors occasionally appear in the specimen, which, if removed and prepared for section and staining, on examination may clear up an obscure diagnosis.

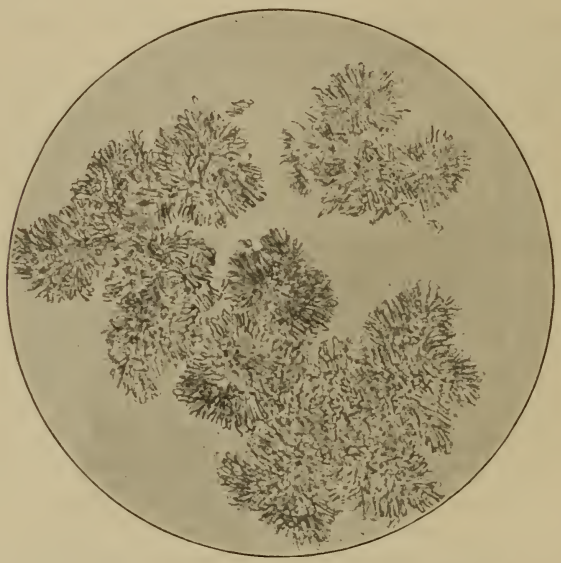


FIG. 7a.—ACTINOMYCES SHOWING RADIAL FORMATION.

Pulmonary Actinomycosis.—This is a rather rare disease caused by the ray-fungus or actinomyces (see Fig. 7a). The characteristic yellowish or grayish-green granules, if found, are often sufficient for a diagnosis; their composition should, however, always be confirmed by microscopic search. In some cases the characteristic microscopic rosettes with clubbed rays are found; in others only branching threads, staining by Gram's method, will be found. These may be confused with atypical forms of the tubercle bacillus.

Echinococcus.—Rarely echinococcus hooklets enter the pulmonary tract and appear in the sputum. They usually originate

in abscesses of adjacent organs, particularly the liver. (See section, "Parasites," page 154.)

Distomum Pulmonale; Syn. Distomum Westermanni.—This organism (for classification see page 102) is a not uncommon cause of disease of the lung in eastern countries, particularly in Asia, but may occasionally be encountered in other parts of the world. The symptoms are not unlike those of pulmonary tuberculosis, for which it may be mistaken. Its presence is determined by finding the ova in the sputum. These are oval, of a brownish-yellow color, with a fairly thin shell, and measure 0.0875 to 0.1025 millimeter in length, and 0.052 to 0.075 millimeter in breadth.

PREPARATION OF THE STAINED SPECIMEN.

Any suspicious particles should be removed from the mass of sputum, and transferred to and carefully spread upon clean slides. These should then be treated to fixation and staining, the technic of which will depend upon the nature of the information sought.

Microscopic Examination of Stained Specimens.—From the standpoint of clinical medicine the most important microscopic object for which search is made is the tubercle bacillus.

Staining Peculiarities of the Tubercle Bacillus.—The recognition of the tubercle bacillus depends upon a special method by which they alone are stained. Unstained, they cannot usually be differentiated from the other organisms which may be present. The ordinary methods employed for staining bacteria are not suitable, so that special technic has been devised and is now regularly employed to render their recognition less difficult.

In these methods advantage is taken of the fact that certain substances increase the activity of staining by aniline dyes. With the tubercle bacillus this is accomplished with carbolic acid. Another important point is that these organisms, when once stained, give up their color only with great difficulty, so that agents which will decolorize all other bacteria in the course of a few minutes will have no appreciable effect upon the tubercle bacillus. It is upon these two peculiarities that we rely in differentiating this organism.

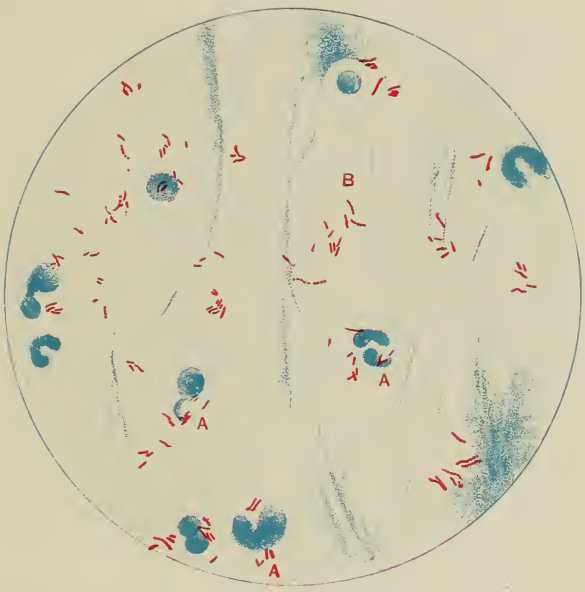
Differential Diagnosis.—While the peculiar micro-chemical reaction toward staining reagents is usually considered to be unique with the tubercle bacillus, it should be remembered that at least three other species of bacteria, when similarly treated, react in the same way. This fact is particularly important in connection with the microscopic examination of urine and pathologic secretions from the genito-urinary tract, and from the rectum. Here is commonly encountered the *smegma bacillus,* which is the next most important member of the group of acid-fast organisms. Acid-fast bacilli have been found in the sputum and about the teeth and tonsils in a case of non-tuberculous disease of the lung.[2]

While these other organisms have the same acid-fast property as has the tubercle bacillus, they appear so seldom that serious mistakes are not likely to occur.

Special Staining Methods.—Ziehl-Nielsen: Place a few drops of the carbol-fuchsin solution (for preparation of stain see Appendix) upon the fixed cover-glass preparation, and hold over a low Bunsen flame until steam begins to rise. Do not boil. Continue the steaming process for from three to five minutes, pour off excess of stain, and wash in water. It is important to prevent the staining reagent from reaching the under surface of the cover-glass. If this is permitted the pigment will dry and become burned into the glass, when it will successfully resist all efforts at complete decolorization. Decolorize with acid-alcohol solution (see Appendix) or with 25 per cent. sulphuric acid. Decolorization should be continued for not less than ten minutes or until the red color has entirely disappeared from the specimen; if this process is not thoroughly done the finished slide, so far as its diagnostic value is concerned, will be worthless. The specimen is then washed in water, and counter-stained for from one to three minutes with a 1 per cent. aqueous solution of methylene-blue, after which it is washed, dried, and mounted for microscopic examination. By this method the tubercle bacilli (see Plate I) will appear as characteristic red rods upon a blue background. A field which reveals other red objects has been insufficiently decolorized and should be discarded for a more carefully prepared specimen.

[2] Pappenheim: Berlin. klin. Woch., No. 37, p. 809, 1898.

PLATE I



Tubercle Bacilli in Sputum.

Gabbett's.—Flood the dried and fixed specimen with carbol-fuchsin and steam for three minutes; then pass directly to the acid methylene-blue stain (see Appendix) for one minute. Finally wash, blot, dry in the air, and mount. This field, if properly prepared, should have the same appearance as that prepared by the Ziehl-Nielsen method, but is not as reliable as the preceding, because a dense spread may resist decolorization, which, owing to the deep-blue counter-staining, may not be noticed until the specimen is mounted for examination.

Pappenheim recommends the following technic: 1. Stain in carbol-fuchsin by steaming near the boiling point for three or four minutes. 2. Pour off the excess of carbol-fuchsin and treat without washing with Pappenheim's solution (see Appendix), pouring it slowly three or four times over the preparation, and allow it to drain off. 3. Wash in water, dry, and mount. Duration of entire procedure from three to five minutes.

Czaplewsky's[3].—This method employs the following solution: (*a*) A special carbol-fuchsin (see Appendix). (*b*) Ebner's decolorizing solution (see Appendix).

Technic.—1. Stain, with the aid of heat, for three or four minutes. 2. Decolorize by treating the specimen alternately with Ebner's fluid and distilled water. 3. Counter-stain with methylene-blue for one minute. 4. Wash, dry, and mount in Canada balsam. The chief advantage of this method is said to be that all acid-resisting bacilli but the tubercle bacillus are decolorized, and, therefore, other organisms, such as the smegma, the hay bacillus, etc., will not, in a properly prepared specimen, appear as taking the special stain.

Microscopic Appearance.—The tubercle bacillus is a delicate rod usually appearing in stained specimens with a beaded internal structure. It may be straight, but is usually slightly curved in its long axis. The length is very variable, some being short, others quite long, though they never appear as long threads. The average length varies between 2 and 5 micromillimeters, and is usually very slender.

In sputum the organisms may occur singly or in groups of from three to half a dozen or more.

[3] Hyg. Rundschau, No. 21, 1896.

Clinical Significance.—The finding of tubercle bacilli in the sputum is positive evidence of pulmonary, bronchial, or laryngeal tuberculosis. On the contrary, their absence, after careful search, even, of a number of preparations, cannot be considered absolute negative evidence.

SPECIAL METHODS OF CONCENTRATING SPUTUM TO FACILITATE BACTERIAL EXAMINATIONS.

If, after careful search of suspicious particles taken from the sputum of a suspect, whose clinical history and symptoms are strongly suggestive of the disease, the organism is not found, the following devices may be resorted to:—

A. The whole amount of a twenty-four hours' specimen of sputum, to which no antiseptic solution has been added, should be placed in a porcelain dish or glass beaker, and stirred with a glass rod until quite thin and diffluent. This should then be stood aside for a few hours to settle, when the lowest portion of the fluid is taken up in pipette and transferred to a centrifuge-tube, where it is rotated for from fifteen minutes to half an hour. From the bottom of the centrifuge-tube a small amount of the sediment is transferred to a cover-glass or slide for fixing and staining.

B. A considerable quantity of sputum is mixed with an equal quantity of water, and a few drops of a 10 per cent. solution of sodium hydrate added. This mixture is then heated until homogeneous, when it is cooled, sedimented, and centrifuged, and examined as outlined above.

ISOLATION OF GRANULAR FORMS OF TUBERCLE BACILLI.

Some observers have found that some specimens after a most careful concentration may fail to show the characteristic organisms by the differential stains, yet they may still, by a more or less elaborate technic, be isolated. These are the so-called granular forms of tubercle bacilli, which are considered degenerative products of the common forms of the organism.

Seelas,[4] however, states that he has never missed finding bacilli themselves in cases where the "granules" can be detected.

4 Semaine médicale, April 30, 1913.

In examining for the granules he adds to the sputum 2 drops of 10 per cent. sodium hydrate solution, shakes a glass-stoppered bottle, heats to boiling, adds one-third part of 50 per cent. alcohol, and allows the mixture to stand in a conical glass for a few hours, after which the upper two-thirds are decanted and the remainder subjected to centrifugation at 4000 revolutions a minute. The flocculent deposit is then placed on slides previously cleansed with potassium permanganate, concentrated sulphuric acid, and absolute alcohol. Two slides are slid one over the other until the deposit has become dry. Fixation is then secured and the specimen stained by the Ziehl-Nielsen method. The staining is done with the slides on a warm copper plate. Finally, the preparations are washed in running water, and counter-stained with Loeffler's methylene-blue; both the tubercle bacilli and "the granules" will appear as red bodies on a blue field.

Antiformin Method.—The most notable advance in the microscopic examination of sputum for tubercle bacilli is the development of the antiformin process of Uhlenhuth.[5]

Antiformin is a 10 per cent. solution of sodium hypochlorite containing from 5 to 10 per cent. of sodium hydrate. This preparation may now be obtained as a proprietary in the open market.

The technic originally advised by Uhlenhuth has undergone slight modifications since it was first published. The technic of E. Burvil Holmes (personal communication) is as follows:—

"If the specimen of sputum is not large I mix it with an equal quantity of a 20 per cent. solution of antiformin in any sterile receptacle, usually a large test-tube or, better, centrifuge tube. This is thoroughly shaken until all the nummular masses are dissolved. The mixture will take on a brownish color when this is complete. A little alcohol (95 per cent.) is now added, as I have found that without it, owing to, I presume, the different densities of the two substances, the bacteria are not well thrown down. The tube is then placed in an electric centrifuge and centrifuged for ten minutes at high speed. The mixture, excepting that at the very bottom, is pipetted off, and some normal

[5] Berlin. klin. Med., August 29, vol. v, 35.

NaCl is added to the small portion at the bottom of the tube and this is thoroughly mixed and again centrifuged for five minutes. Again all except the very bottom, which is placed on a clean glass slide upon which preferably a little egg-albumin has been smeared, is pipetted off. The slide is stained in the usual way."

Another modification is that advocated by Webster,[6] which is Loeffler's[7] modification of the original antiformin process. The technic is as follows: 5, 10, or more cubic centimeters of sputum are placed in a flask and mixed with an equal quantity of a 50 per cent. solution of antiformin (a 10 per cent. solution of sodium hypochlorite containing 5 to 10 per cent. of sodium hydrate) and boiled for a period not exceeding fifteen minutes. Solution occurs associated with considerable foaming and browning of the mixture. For every 10 cubic centimeters of this solution are now added 1.5 cubic centimeters of a mixture of 1 part of chloroform and 9 parts of alcohol. After thoroughly shaking to produce a fine emulsion, portions of the fluid are placed in sedimenting tubes, the tubes are corked, and centrifuged for fifteen minutes. The heavier elements collected in a film just above the chloroform, which film holds the tubercle bacilli, owing to the marked affinity of chloroform for the fatty and waxy material in these organisms. The supernatant liquid is poured off and the film above mentioned is removed and placed upon a glass slide, the excess of fluid being taken up with filter-paper. As a fixative a drop of egg-albumin, preserved with ½ per cent. carbolic acid, is added and a thin spread made by means of a second slide. This smear is allowed to dry and is then stained by one of the usual methods.

This enrichment process of Loeffler furnishes preparations which often show a remarkable increase in numbers of tubercle bacilli as compared with those found by the usual smear methods. A further advantage of this method is that practically all organisms, with the exception of those of the acid-fast type to which the tubercle bacillus belongs, are destroyed. This permits one to obtain material (omitting, of course, the application of heat) for pure-culture work or for inoculation

[6] "Diagnostic Methods," P. Blakiston's Sons, Philadelphia, 1912.
[7] Deutsch. med. Wochen., Bd. 36, 1910, S. 1987.

purposes, which will be free from the secondary invaders which so often interfere with the establishment of an absolute diagnosis. Naturally, in the study of the mixed infections in tuberculosis one should also examine preparations made in the usual way.

Method of Ellermann-Erlandsen.[8]—Another method for concentrating tubercle bacilli in a specimen is H. Kogel's modification of the so-called double method of Ellermann-Erlandsen and is as follows: (1) One volume of sputum (10-15 cubic centimeters) is mixed in a stoppered glass bottle with one-half its volume of 0.6 per cent. sodium carbonate solution. The mixture now stands twenty-four hours in the thermostat at 37° C. (2) The greater part of the supernatant fluid is decanted and remainder is centrifugated in a graduated centrifuge tube. The fluid is poured off. (3) Four volumes of 0.25 per cent. sodium hydroxide are added to one volume of the precipitate. After very carefully agitating, this is raised to boiling. (4) Centrifuge and make smear of the sediment.

The result of this treatment is that practically the entire bacillary content of the whole amount of sputum is spread on one or two slides and 20 to 30 times the number of tubercle bacilli occur per field. The method takes time. In an old sputum autodigestion may have gone far enough to make the first stage of the procedure unnecessary. Very thick sputa may require 48 hours in the thermostat. Very purulent sputa give poorer results than slimy ones. These must be left longer in the incubator and must be boiled longer, with larger amounts of the caustic. A powerful centrifuge is required, and the centrifugation must be continued long enough to precipitate completely all the solid matter. The final precipitate consists almost entirely of bacteria. A glance is often all that is required for a diagnosis. Specimens which in the usual smear showed 10 tubercle bacilli to the field, by the double method showed 300 to 400. As a rule, 15 to 30 times as many may be seen. Of 105 specimens of sputum examined by the usual method, 21 were positive. Of those negative, 8 by the double method gave positive results, or an increase of 8 per cent.

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[8] Deutsch. med. Wochen., Dec. 2, 1909.

Much's Method of Staining.—Among the newer methods of staining tubercle bacilli is the method of Much, which has been carefully studied by Liebermeister,[9] who says that certain tubercle organisms not demonstrated by the usual methods may be shown by a prolonged Gram stain; the tubercle bacilli being Gram-positive though they are not absolutely acid-fast. The acid-fast types of tubercle bacilli are distinctly granular and frequently appear as mere "granules" rather than true bacilli (see page 34). These "granules" under certain unknown conditions change into true bacillary types and *vice versâ*. Smears are prepared in the usual way or by one of the special methods above described, and are then treated as follows: Cover the smear with carbol-methyl-violet solution (Much's stain, see Appendix page 407), and heat to boiling several times. Wash stain off with water and cover smear with Lugol's solution for five minutes. Wash with water and treat with 5 per cent. nitric acid for one minute and follow this with 3 per cent. hydrochloric acid for ten seconds. Without washing place the slide in a mixture of equal parts of acetone and absolute alcohol until the smear is colorless. Wash with distilled water and counter-stain with 1 per cent. aqueous solution of saffranin for a few seconds. Wash in water, dry thoroughly and examine with immersion lens. The tubercle bacilli and the granular forms appear blue, while the other organisms are red.

Spengler's technic is fairly simple and is applied to the dried film as usually prepared:—

1. Stain with carbol-fuchsin, warm, but without too much heat. 2. Pour off the stain without washing. 3. Pour on picric acid alcohol (consisting of equal parts of saturated aqueous solution of picric acid and absolute alcohol). After three seconds: 4. Wash with 60 per cent. alcohol. 5. Treat with 15 per cent. nitric acid till yellow (thirty seconds). 6. Wash again with 60 per cent. alcohol. 7. Counter-stain with picric acid alcohol till lemon-colored. 8. Wash with distilled water and dry gently at a low heat.

The bacilli appear bright red on a lemon-colored ground, and, if present, are more perceptible than by any other method. With a little practice this will be found an easy and fairly

[9] Deutsch. med. Wochen., July 15, 1909.

rapid method, and the extra time involved in the process will be more than compensated by the ease with which the bacilli will be found, if present.

MICROCOCCUS LANCEOLATUS.

The presence of Fränkel's diplococcus in the sputum of patients suffering with croupous pneumonia is fairly constant, so that its demonstration is of considerable diagnostic importance. They appear as elongated lanceolate cocci, usually arranged in pairs with their bases approximated. They are surrounded with a faintly staining capsule which, in dry preparations, does not usually take the stain at all, although the ordinary method employing methylene-blue has occasionally, in the author's experience, demonstrated a faint but distinct capsule.

The organism is supposed to be the cause of lobar pneumonia, but must not be confounded with the other diplococci occurring in the sputum, more especially with Friedländer's bacillus. The latter also possesses a capsule, but has nothing whatever to do with the production of lobar pneumonia, though occasionally they may accidentally be present. Friedländer's organism, when highly magnified, will be found to be a short rod. Cultural characteristics will also serve to differentiate, as will also Gram's staining method, which decolorizes Friedländer's and stains Fränkel's organism.

The following modification of GRAM'S METHOD will be found satisfactory (for preparation of staining reagents see Appendix): Hold the fixed and dried cover-glass preparation in the forceps and flood with carbol-gentian violet; allow this stain to act for from three to four minutes. Wash and transfer to the iodine-potassium-iodide solution for from one to two minutes. Next wash in alcohol until the apparently dirtily stained film is decolorized. Transfer to absolute alcohol, then to oil of cloves, and finally mount in balsam. By this method the organism appears as a dark-blue or violet diplococcus. Friedländer's organism will remain unstained.

A very useful method for differentiating Fränkel's coccus is that devised by W. Wolf. By this method the dry preparation is first stained in aniline water saturated with fuchsin, and is then placed for one or two minutes in a dilute watery solution

of methylene-blue. The cocci will now be found stained blue, the capsule rose-red, and the body of the specimen purplish red.

Method for Staining Capsule.—Prepare the cover-glass smear in the usual way, then without drying flood with glacial acetic acid. At the expiration of one minute pour off the excess of acid, and without washing flood the specimen with aniline water gentian-violet (Koch-Ehrlich), which should be allowed to act for four minutes, when the excess of stain is poured off and a fresh portion added, which is allowed to act for another two minutes. The cover-glass is now washed in two or three changes of normal saline solution (it may be found necessary to employ a saline solution of 1.5 to 2.0 per cent.), after which it is blotted, dried, and mounted in the usual way.

By this method, the capsule will appear as a faintly tinted halo about the diplococcus. Clinically the absence of Fränkel's micro-organism practically excludes the diagnosis of lobar pneumonia, although its demonstrated presence is by no means positive evidence in the other direction, because this organism has repeatedly been demonstrated in the sputum and in the mouth secretions of healthy individuals.

BACILLUS OF INFLUENZA, OR PFEIFFER'S BACILLUS.

A small, slender bacillus occurring usually in very great numbers in the nasal secretion of fresh attacks of true influenza, but not found in the ordinary short attacks of prostration accompanied by coryza, which is at present designated influenza or "grippe," largely for lack of a more definite diagnosis. *Morphologically,* it is a very small rod appearing frequently in pairs; in parts of the secretion this organism may be found in what is practically a pure culture, occurring both within and without the leukocytes. Their length is usually from two to three times their width; they rarely form chains. The ends of the rods are rounded, and if imperfectly stained (they stain with difficulty) the ends will be more deeply stained than the center, giving an appearance not unlike a diplococcus with just a suggestion of a capsule. According to recent authority, this bacillus does not possess a capsule, the deceptive appearance being probably a staining peculiarity of certain cells. The bacillus is non-

motile, and can only be cultivated upon special media containing hemoglobin. This characteristic will readily serve to differentiate it from the colon bacillus and other organisms of similar appearance. An easily made and satisfactory medium is prepared by spreading a little fresh blood upon the surface of an ordinary agar slant and inoculating this with the infected material. This organism only develops on artificial media between the temperatures of 26° and 43° C., growing best at body-temperature. Upon the blood-agar slant incubated at 37° C., there will develop minute, transparent, watery colonies that are without structure, somewhat resembling droplets of dew. They are usually discrete, and show little or no tendency to coalesce.

Staining.—One of the best methods of staining is with a dilute watery solution of Ziehl's carbol-fuchsin (the color of the solution should be pale red). This solution should be allowed to act for five minutes. This organism is decolorized by Gram's method.

A second method of staining is with Loeffler's methylene-blue (for preparation see Appendix). This stain should be allowed to act for five minutes. Then wash in water, mount, and examine for blue organisms.

LEPRA BACILLUS.

The bacillus lepræ, first described by Hansen, is a small, slender bacillus from 4 to 6 microns in length and surrounded by a slimy envelope. These bacilli behave toward staining reagents very much like the tubercle bacillus, but are less resistant toward acid and alcohol than is the tubercle bacillus, so that a differentiation is possible, provided decolorization is rather severe. The stained bacillus often shows clear spots or appears as if made up of stained granules.

Methods of Staining.—Any of the special stains employed in examining for the tubercle bacillus may be used. Of these that of Pappenheim is very satisfactory, because, when decolorization is complete, nothing retains the red pigment but the tubercle bacillus. Such staining methods applied to the lepra bacillus are at best of negative value.

These organisms may be found in many cases of leprosy, in the sputum or in the nasal secretions, so that in doubtful cases

a differentiation is necessary. It may be necessary to resort to inoculation experiments to make an absolute differentiation.

THE BACILLUS PERTUSSIS.

This organism, discovered by Bordet and Gengou, and elaborated by Klimenko, has been frequently found in the sputum in cases of whooping-cough. It resembles very closely the influenza bacillus, appearing as short, plump, ovoid bacilli, with rounded ends, lying singly or in small groups between the pus and epithelial cells. It stains feebly with the usual dyes and is Gram-negative. This organism is rarely intracellular and may thus be distinguished from the influenza bacillus.

SPECIAL REACTIONS APPLIED TO THE SPUTUM.

In recent years several chemical tests and reactions have been applied to the sputum, as further aids in diagnosis, some of which have already attained a practical value.

Pacini's Color Reaction in Pneumonia.—A. P. J. Pacini[10] describes a reaction persistently observed in the sputum of pneumonia patients which, after examination of 1200 specimens where the ultimate diagnosis was confirmed and established as pneumonia, showed an error of only 2 per cent.

Because of the combined accuracy. and simplicity of the reaction, it should prove useful to the busy general practitioner.

This test is applied as follows: A portion of the sputum is mixed with distilled water in the proportion of one volume of sputum to ten volumes of water, and agitated in a suitable container for five minutes. The mixture is filtered through paper and preserved for the test.

A 1 per cent. aqueous solution of methyl-violet constitutes the reagent necessary for this reaction, which should be prepared as stock and kept ready for use.

To a test-tube containing 10 cubic centimeters of distilled water add 5 drops of methyl-violet solution and mix thoroughly. Then add, drop by drop, 10 drops of the filtrate obtained as above described.

In the event of a positive reaction, the methyl-violet

[10] The Interstate Medical Journal, June, 1913, p. 536.

assumes a distinct red color. Nothing short of a red color constitutes a reaction.

This reaction is present only in the sputum of patients at the onset of or during an attack of pneumonia. It is due to a specific disintegrated blood pigment characteristically present in the sputum of such patients, and precedes the expectoration of the classic "rusty sputum" by several days.

Test for the Albumin Content of the Sputum.—Beginning with the work of Rodger and Levy-Valanci,[11] and since repeated by others, it has been found that the sputum of tuberculosis in nearly every case, irrespective of age, gives a positive albumin reaction, which substance is not normally present in the sputum. It is now believed that the demonstration of albumin in the sputum is a most important diagnostic aid in the demonstration of the earlier phases of pulmonary tuberculosis, even before the appearance of the specific bacilli in the sputum. No direct relation between the number of tubercle bacilli present and the albumin contained has yet been demonstrated. Some cases give negative reaction and fail to show the germs, after most careful examination, and *vice versâ*.

According to Dr. John Ritter,[12] a good technic is as follows:

Test for Sputum Albumin.—A proper way for collecting sputa is most essential to the correct interpretation of the albumin reaction. First wash out the mouth or gargle with a few mouthfuls of warm water to clear the mouth and throat from all adherent secretions, after which the sputum should be collected. The patient must be instructed to cough up deep from the lungs, usually in the early morning, the first on rising, and place the sputum in a clean sterile bottle and securely close it with a well-fitting cork stopper. He must be specifically instructed not to collect the secretions from the mouth, buccal cavity, nasopharynx, or from the throat, but only such as he can bring up from the bronchial tubes, for this alone is suitable for making a reliable albumin test. A properly collected specimen should be examined at once; in any event it must not be more than about six hours old; in cold weather an expectorated sample will keep for twenty-four hours before undergoing fermentative

[11] Presse médicale, No. 32, 1910; No. 40, 1911.
[12] Medical Record, April 26, 1913.

changes. Only the freshly collected sputum should be tested; decomposed secretions will often give a reaction simulating albumin. No antiseptic solution or any preservative agent should be added before making the albumin test.

Technic.—Put 5 cubic centimeters of sputum into a glass cylinder of about 25 cubic centimeters' capacity (glass cylinder must be supplied with a well-fitting ground-glass stopper), add 5 cubic centimeters of distilled water and about 10 drops to 1 cubic centimeter of glacial acetic acid, replace the stopper, shake well, and set aside. Shake occasionally during the next twenty or thirty minutes, then proceed to filter. If the sputum is suitable for filtration it will have separated into three distinct layers, reminding one very much of an expressed stomach content ready for testing. Should the middle or watery portion of the sputum solution be still a little opaque, showing the presence of some remaining unprecipitated mucin, add a few drops more of the glacial acetic acid, again shake well, and set aside as before. The fluid portion now appearing clear, proceed to filter through a plain wet filter and test the clear filtrate for albumin in the usual way by either Heller's test or the heat test.

Rivalta's Acetic Acid Test for Albumin in Sputum as Modified by Casali.[13]—Because of its simplicity this method has much to recommend itself to the busy practitioner. The technic of this test is as follows: Ten cubic centimeters of sputum are thoroughly stirred with 10 cubic centimeters of distilled water and then slowly filtered. Two drops of acetic acid are stirred into a glass containing 100 cubic centimeters of distilled water. Holding the glass before a light, a drop of the filtrate is cautiously dropped into the fluid. In case of a positive reaction, the drop becomes surrounded with a little cloud as it falls through the fluid. By graduated dilution of the filtrate with a weak solution of sodium carbonate (1 drop in 100 cubic centimeters of water), the test can be carried to its extremest limits. The significance of albumin found by this test is the same as in the preceding.

[13] Riforma Medica, Naples, July 27, 1911, No. 30.

III.

THE BLOOD.

GENERAL CONSIDERATIONS.

The blood is the most important fluid of the body, as it bears a more or less definite and direct relation to all other body fluids and tissues. It is the intermediate substance between the nutritive materials taken into the body through the digestive tract and elaborated into pabulum by it, and body cells, to which it both gives nutriment and carries away effete and waste materials.

We must, therefore, have a working knowledge of the normal physiology and histology of the blood, in order to comprehend departures from the normal and to appreciate their pathologic significance.

There are but few definite diagnostic findings which can be based upon hematologic examination alone; nevertheless, there are an infinite number of conditions in which a study of the blood, from one or more standpoints, will shed valuable light, which will often give material aid in an obscure diagnosis. It must be remembered that both physiologic and pathologic conditions may influence both the quantity and the quality of the blood. This is becoming more and more evident as the mysteries of the plasma are being brought to light through recent systematic studies of the agglutinins, opsonins, lysins, precipitins, the phenomenon of anaphylaxis, etc.

It is beyond the scope and purpose of this book to cover completely the whole subject of clinical hematology, and no effort has been made to so do; any omissions encountered by the student will have to be augmented by collateral reading from one of the recent works on the blood. The author's intention has been, in the preparation of this section, to furnish the active practitioner and student with a safe practical guide to the performance of the more commonly used clinical methods.

Technic requiring considerable time for its performance, or complicated apparatus, has largely been eliminated, while, as a matter of convenience, sections on the coagulation time and viscosity have been included under the general head of the blood.

PHYSICAL AND CHEMICAL PROPERTIES.

Appearance of Fresh Blood.—The exuding drop of blood shows even to the naked eye a number of properties. The redder it is, the richer it is in oxyhemoglobin; the darker, the greater the amount of reduced hemoglobin. Microscopically, it reveals a great number of cellular elements; these are colored and colorless discs.

The *red cells* appear as non-nucleated biconcave discs, measuring on the average 7 micromillimeters in diameter. Viewed singly through the microscope by transmitted light, they are of pale-greenish hue. The *colorless cells* or *white corpuscles* are, as a rule, somewhat larger than the red cells, and present either mono- or poly- nucleated protoplasm.

The plaques, or *blood-platelets,* appear as minute, colorless discs measuring less than half the diameter of the red cells. They usually occur in groups or bunches of half a dozen or more, and are present in normal blood to the number of from 225,000 to 350,000 per cubic millimeter.

There are no other morphologic constituents of the blood.

Color.—The color of normal blood is due to the presence of an albuminous substance in the corpuscles termed hemoglobin. In the arterial blood it is in combination with oxygen, and is here termed oxyhemoglobin. In the venous blood a mixture of both hemoglobin and oxyhemoglobin occurs. With a preponderance of oxyhemoglobin, the blood tends to a scarlet hue; when the hemoglobin predominates, the blood is of a bluish color.

Pathologic Changes in Color.—In coal-gas poisoning the blood is cherry-red. After poisoning from potassium chlorate, aniline, hydrocyanic acid, and nitrobenzol, the blood is brownish-red or chocolate color. In extreme cases of leukemia the blood may have a milky appearance due to the excessive number of white blood-cells present.

The Odor.—This is characteristic and differs in different species of animals. It is due chiefly to the presence of volatile fatty acids.

The Taste.—The taste of blood is salty, but at the same time insipid.

The Specific Gravity.—The specific gravity seems to vary with the amount of hemoglobin. It is influenced by the age and sex of the individual, the process of digestion, exercise, pregnancy, etc. The normal average in adults varies between 1.058 and 1.062.

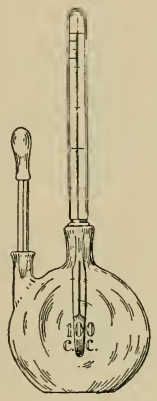


FIG. 8.—PYCNOMETER.

METHOD BY PYCNOMETER.—The most accurate instrument for determining the specific gravity is the pycnometer. This method is open to the objection that it requires much more blood (10 to 50 cubic centimeters) than can usually be obtained in routine work. In cases in which bleeding can be resorted to without detriment to the patient, this is the method to use.

The technic is as follows: Weigh the pycnometer (Fig. 8) three times, on an accurate clinical balance: (*a*) empty; (*b*) filled with distilled water, and (*c*) filled with blood. Care should be taken to have the vessel absolutely dry and clean before weighing it empty and before filling with either water or blood. Subtract the weight of the empty bottle from that of the bottle filled with blood and divide this figure by the difference in weight between the bottle filled with water and the empty bottle. The

result will be the specific gravity of the blood, water being taken as unity. In this determination it is essential, in order to insure accurate results, to have the temperature of the water the same as that of the blood.

Method of Hammerschlag.—A cylinder about 10 centimeters in height is partly filled with a mixture of benzol (sp. gr. 0.889) and chloroform (sp. gr. 1.526), so that the specific gravity of the mixture lies between 1.050 and 1.060. Into this a drop of blood is allowed to fall directly from the finger. It is then brought into suspension by the addition of either a little chloroform or benzol, according to the tendency of the drop to sink or rise in the cylinder.

As soon as the drop remains stationary in the fluid the specific gravity of this is taken by an accurate hydrometer (one reading to the fourth decimal should be used) or, better, with a Westphal balance. The reading represents the specific gravity of the blood tested.

The Amount.—The total amount of blood in the normal adult is said to amount to about one-twelfth or one-fourteenth of the body weight.

The Reaction.—The reaction of the blood is slightly alkaline, due to the presence of the monosodium carbonate and the disodium phosphate in solution in it. The reaction may be roughly determined by drawing a strip of neutral litmus paper, which has been thoroughly moistened with a concentrated solution of common salt, through the blood, and then rapidly washing the corpuscles off with the same solution. Owing to the development of certain acids, the alkalinity of the blood rapidly diminishes after it is shed. This fact renders this determination a rather difficult matter. The normal variation of alkalinity is very slight. By accurate titration the normal degree of alkalinity of the blood, under normal conditions, corresponds to from 325 to 360 milligrams of sodium hydrate for every 100 cubic centimeters of blood.

THE CHEMICAL COMPOSITION OF THE BLOOD.

A general idea of the composition of the blood may be had from the following table, which is taken from Simon's

"Physiologic Chemistry." The calculations are made for 1000 parts by weight.

Corpuscles	480.00	parts
Water	276.90	"
Oxyhemoglobin	193.90	"
Stroma, including salts	9.20	"
Plasma	520.00	parts
Water	477.37	"
Albumins	35.88	"
Extractives	2.39	"
Inorganic salts	4.36	"

The predominating solid substance in the blood is oxyhemoglobin; it represents 10 per cent. of the total weight of the blood, 40 per cent. of the weight of the corpuscles, and 65 per cent. of all organic matter present.

The mineral constituents comprise sodium, potassium, calcium, magnesium, and iron.

Fats are present to the extent of from 0.2 to 0.3 per cent. These may be temporarily increased after the ingestion of much fatty food, and also in many pathologic conditions.

The plasma normally contains small amounts of oxygen and nitrogen in solution, with varying amounts of carbon dioxide.

METHODS OF OBTAINING BLOOD FOR EXAMINATION.

The tip of the finger and lobe of the ear are the sites usually selected from which to obtain specimens. In the majority of examinations only a small amount—a few drops—is necessary. This is obtained by simple puncture of the skin made with a Glover needle, the half-point of a new steel pen, a *Daland* lancet (see Fig. 9), or the so-called pistol-knife. The last-mentioned two instruments are to be preferred because they permit of regulation of the depth of the puncture.

For hospital and laboratory use, in order to assure a sterile instrument for each patient, I have, for a number of years, employed short sections of thin glass tubing, drawn out in the flame to a capillary point, and then broken so as to have a short, sharp end. There is little danger, if these are properly

made, of leaving fragments of glass in the tissues. After using, these may either be thrown away or retipped in the flame.

For larger quantities of blood, a few cubic centimeters or more, it is advisable, provided there is no contraindication, to obtain the specimen by the use of wet cups. It must be borne in mind that by this method there is always more or less admixture of lymph. Another method is to draw the blood from



FIG. 9.—FLEISCHL HEMOGLOBINOMETER, SURROUNDED BY ACCESSORIES NECESSARY TO PERFORMANCE OF THE TEST, INCLUDING DALAND LANCET.

a dilated vein into a large sterile antitoxin syringe. Lastly, an ordinary venesection may be resorted to.

The withdrawal of blood, if aseptically performed, is practically free from danger and need disturb the patient very little. Before proceeding in any case it is advisable to determine the absence of hemophilia in the patient.

CLINICAL METHODS.

The clinical methods commonly applied to the blood in the study of disease are: The estimation of the percentage of

hemoglobin; the enumeration of the erythrocytes and the white blood-corpuscles; a differential count of the various white elements; estimation of the coagulation time and of the viscosity.

The apparatus necessary for the performance of these several examinations are as follows:—

I. A good microscope with the ⅓, ⅙, and oil-immersion (1/12) objectives.

II. A hemoglobinometer.

III. A hemocytometer.

IV. A coagulometer.

V. A viscometer.

VI. Slides, covers, stains, etc. (for complete list see Appendix).

ESTIMATION OF THE HEMOGLOBIN.

Method of Gower.—Gower's hemoglobinometer consists of: (*a*) Two glass tubes, one of which contains a standard solution of picrocarmine in glycerin jelly of a color equal to a 1 per cent. solution of normal blood. The other tube is graduated in percentage up to 120. (*b*) A graduated capillary pipette measuring 20 cubic millimeters with a rubber filling tube attached. (*c*) A long, fine glass pipette with rubber bulb for diluting the blood with distilled water.

The standard and graduated tubes are flattened on two sides, and for comparison are placed in a rubber stand. A white light should be used, which should be made to shine through a sheet of white paper during the comparison of the colors.

Technic.—Twenty cubic millimeters of blood are sucked up into the graduated pipette and blown into the graduated tube; distilled water is then drawn up to the same mark and blown into the graduated tube; by this means the capillary pipette is cleaned and no hemoglobin lost. Then by means of the other pipette, distilled water is added, drop by drop, to the blood, shaking it frequently, until the color corresponds with that of the standard. The number on the graduated scale at the level of the diluted blood, when the two tubes are of the same tint, represents the percentage of hemoglobin.

This instrument is accurate between 2 and 3 per cent., but below a reading of 10 per cent. it is very difficult to judge with

certainty any difference in the tint. This may be overcome by employing two or more pipettes full of blood and then dividing the results by the number of pipettes (20 cubic millimeters each) employed.

Method of Fleischl.—The instrument consists of a metal stand having a horizontal table with a circular aperture in its center, beneath which is placed a reflector of plaster-of-Paris. Immediately beneath the aperture and above the reflector, a graduated wedge of tinted glass is arranged to move in a horizontal plane by means of a milled thumbscrew. This graduated wedge of glass is shaded to represent varying degrees of hemoglobin content, and carries with it a scale indicating the blood-strengths. For collecting and diluting the blood for examination, a pipette and diluting chamber are furnished. *The technic* is as follows:—

By the aid of the pipette which is furnished with the apparatus, an exactly determined amount of blood is dissolved in a measured quantity of distilled water (the contents of one-half of the diluting chamber, the other half being filled with an equal amount of distilled water).

As the capillary pipettes vary in size for each chamber accompanying apparatus, it is essential, when purchasing new pipettes, to obtain those having a number on the metal handle the same as on those originally accompanying the instrument; otherwise, the observation will be incorrect. The cell thus prepared is then placed over the aperture in the table, and artificial (candle, lamp, or gas) light is directed through it by means of the reflector. The part of the wedge is now searched for which accurately compares in tint with the solution of blood under examination, and the number of the scale is read off that corresponds to this point on the glass wedge. (It is a matter of common experience that the grading of these instruments is too high, and that a specimen of blood that corresponds to 90 or 95 on the scale is normal.)

Hemoglobinometer of Fleischl-Miescher.—A recent improvement in the Fleischl hemoglobinometer has been made by Miescher. This instrument (Fig. 10) is similar in general appearance to the original Fleischl. It has the same stand and the same scale principle, although this latter is standardized

differently and graduated on a different basis. It differs materially in the method of measuring and diluting the blood, in the form of the comparison chamber, and in the meaning of the graduation of the scale.

The diluting pipette is similar in construction to the pipette of the Thoma-Zeiss hemocytometer, its calibrations, however, being different. The marks are ½, ⅔, and 1. Above

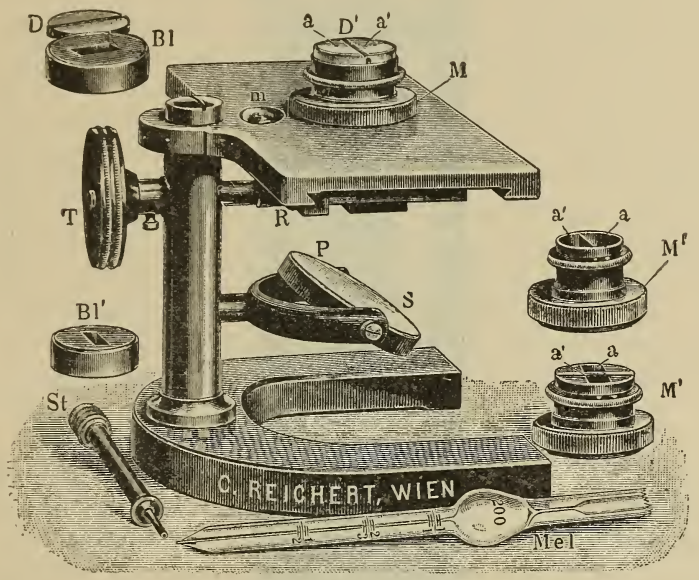


FIG. 10.—FLEISCHL-MEISCHER HEMOGLOBINOMETER.

and before each of these main divisions are two marks, each corresponding to $\frac{1}{100}$ of the contents of the capillary tube. This device enables the worker to measure accurately the column of blood taken, in case he gets too little or too much blood in the tube. The relation of the capillary to the ampulla is such that blood, drawn to the mark 1 and diluted to the mark above the ampulla, gives a dilution of 200; if drawn to the mark ⅔, the dilution is 300, while the line ½ furnishes a dilution of 400. The diluent used is $\frac{1}{10}$ per cent. sodium carbonate solution. This dissolves the stromata of the red cells and produces a clear solution. If the diluent becomes turbid

after standing some time, it should be freshly made and should contain no bicarbonate.

Calculation of Results.—This is possible only with the use of the "table of calibrations," which contains the series of scale divisions and the absolute amount of hemoglobin in milli-

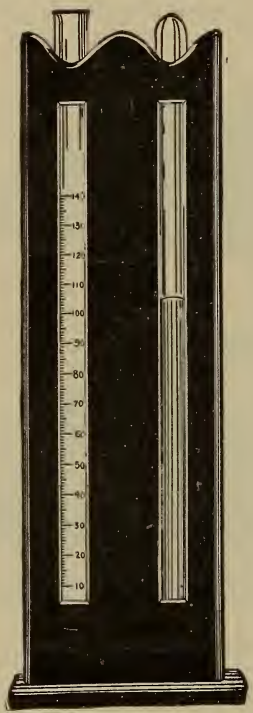


Fig. 11.—Sahli's Hemoglobinometer.

grams per 1000 cubic centimeters of blood, corresponding to each division of the scale when the 15-millimeter chamber is used.

The principle of Sahli's hemoglobinometer (Fig. 11) is taken from Gower's hemoglobinometer, but contains a more permanent standard solution. The latter is made by the action of hydrochloric acid upon blood, so that hydrochlorate of hematin is produced, which serves as the standard color. In

tint it corresponds to a 1 per cent. solution of normal blood. The blood to be examined is also acted upon with a definite solution of hydrochloric acid ($\frac{1}{10}$ normal) and the color altered by reason of a similar formation of hydrochlorate of hematin varying in intensity according to its hemoglobin content. Thus two solutions of the same color are compared. The blood solution is mixed in a glass tube graduated to represent percentages of hemoglobin. Twenty cubic millimeters of blood are drawn into a pipette similar to that of Gower's; this is blown into the graduated tube, which has been filled to the mark 10 with the hydrochloric acid solution. When the mixture has become a clear dark-brown color, distilled water is added until it corresponds to the standard color solution, and the percentage of hemoglobin is read off on the scale. The two tubes are fitted into a black vulcanite holder which acts as a light screen and which is backed by a ground-glass plate for a light diffuser. The comparison is made by transmitted light, the result being the same whether it be made in artificial or in day light.

Some years ago there was some difficulty experienced in obtaining a reliable Sahli instrument; for this reason the author has not since recommended it.

Method of Dare.—This instrument (Fig. 12), introduced by Dare, has the advantage of using undiluted blood; hence it avoids any error consequent upon dilution. The principle of this instrument is as follows: The color of undiluted blood is compared by artificial light with that of a graduated glass scale made of ruby glass (purple of Cassius) the 100 point of which is standardized against a solution of 13.77 grams of hemoglobin in 100 cubic centimeters of serum.

Method.—Swing outward the movable screen, which serves as a cover for the case; adjust the eye-tube, and fit the candle attachment in its place opposite this. The candle should be so adjusted that its upper end is flush with the top of the clasp which holds it. See that the pipette, composed of the rectangular glass plates, is thoroughly cleansed and dry. The space between the plates is filled by applying the edge of the pipette to the side of a fairly large drop of blood. Adjust this pipette in its place and rotate the color scale, by means of the milled screw, until the colors match. Hold the instrument steady to

prevent any flickering of the flame. No dark room is necessary, but it is advisable to point the instrument at some dark object and to avoid direct sunlight, as the shadings of color are not so easily matched by direct day light. As soon as the colors are matched make the readings. This reading is observed on the left side of the case in the small open space,—the line which coincides with the beveled edge of the opening represents the percentage of hemoglobin, on the basis of a value of 13.77

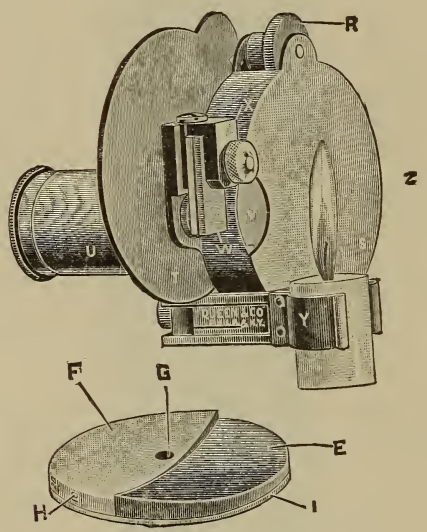


FIG. 12.—DARE'S HEMOGLOBINOMETER.

grams of hemoglobin per 100 cubic centimeters as 100 per cent. It is, therefore, easy to calculate the direct amount of hemoglobin in the blood examination.

This instrument has the advantages that undiluted blood is used, that the scale of comparison is usually very accurately standardized, that it is convenient, easy of manipulation, and rapid in giving results. Coagulation of the blood does not occur sufficiently soon to introduce an error, providing the reading is taken within a reasonable time. It is more convenient for general use than most others, can be used in a light room, but it is rather expensive, and an occasional inaccurate instrument has been found.

ENUMERATION OF THE CORPUSCLES.

Method of Thoma-Zeiss.—The Thoma-Zeiss apparatus consists of a counting slide and two diluting pipettes, termed "melangeurs" (Fig. 13). This slide is so constructed that it contains a chamber in its center having a depth of $\frac{1}{10}$ millimeter. The center of the floor of the cell is divided by fine microscopic lines into minute squares, the sides of which are equal to $\frac{1}{20}$ millimeter. The cell is completed by the application of a special cover-glass. Under these conditions each cube as outlined has a capacity of $\frac{1}{4000}$ cubic millimeter.

These small squares are divided into groups of sixteen by

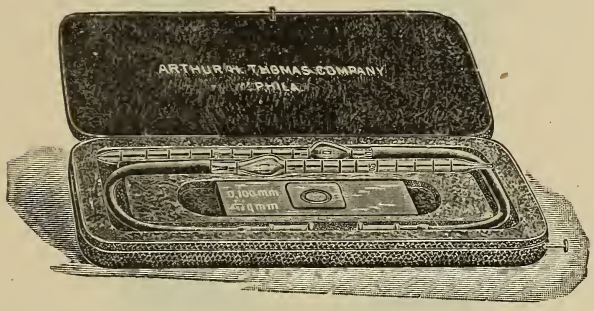


FIG. 13.—THOMA-ZEISS HEMOCYTOMETER IN CASE. (A. H. T. Co.)

means of double ruled lines, each group of sixteen small squares constituting a large square. There are sixteen of these large squares in the slide. There are, therefore, 256 small squares in the 16 large squares. If we include also the small squares forming the boundaries between the large squares, the total number of small squares will be four hundred (Fig. 16). This counting chamber is used in estimating both the red and the white cells; also the number of cells in the cerebrospinal fluid, pleural effusions, etc.

Estimation of the Erythrocytes.—The "melangeur" having the smallest bore, and having marks at 0.5, 1.0, and 101, is used in this estimation. The drop of blood issuing from the puncture is drawn up to the 0.5 mark, the tip then quickly freed from adherent blood, and immersed in a 2½ per cent. solution of potassium bichromate (or Hayem's solution, for

which see Appendix), which is drawn up to the 101 mark. The tip of the tube is now stopped with the finger and the tube vigorously shaken, for at least a minute, to insure thorough and even dilution of the blood. The portion of the diluting fluid contained in the capillary part of the tube is blown out and wiped away, and the next drop of the mixture placed in the center of the floor of the counting chamber. This drop should be entirely free from bubbles. The cover-glass is now applied over this and pressed down firmly around the edges until Newton's rings appear.[1] A few moments should now pass before

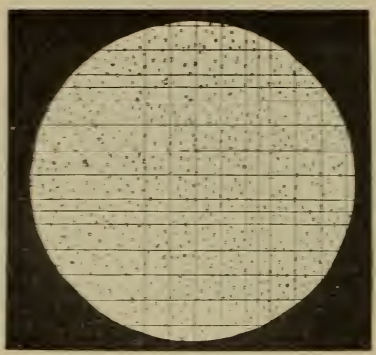


FIG. 14.—APPEARANCE OF FIELD OF THOMA-ZEISS HEMOCYTOMETER WHEN PROPERLY MOUNTED FOR COUNTING THE RED CORPUSCLES.

counting to allow the corpuscles to settle to the bottom of the chamber.

A simple and practical method of arriving at the number of red corpuscles obtained in a cubic millimeter of blood is the following: Select and count from the various parts of the chamber five large squares (Fig. 14), counting the border cells only upon the upper and right-hand lines. The total of small squares counted will be eighty. To the total of the corpuscles counted in the five large squares add four 0000, and the number resulting will be the number of red corpuscles in 1 cubic millimeter of undiluted blood.

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[1] A series of curved, prismatic lines appearing between the plane glass surfaces, where they are firmly pressed together.

Enumeration of the Red Cells.—*Explanatory note:* $\frac{1}{4000}$ cubic millimeter equals the cubic capacity of one small square. $\frac{1}{200}$ equals the dilution of the specimen of blood. Five large or 80 small squares are the number of squares counted. Then the number of cells per cubic millimeter in the undiluted specimen will equal the number of cells in one small square multiplied by the dilution times 4000, viz.: —Let x equal cells per cubic millimeter, and let y equal number of red blood-cells in 80 small squares. Then, $\frac{y}{80} \times 200 \times 4000 = x$, which, simplified, is the same as $y \times 10{,}000 = x =$ the number of red blood-cells in one cubic millimeter of undiluted blood.

A Second Method.—Count all the small squares (400); divide the number of cells counted by the number of squares. This will give the average number of cells in one square ($\frac{1}{4000}$ cubic millimeter) of diluted blood. To determine the number of cells in 1 cubic millimeter of undiluted blood, it is only

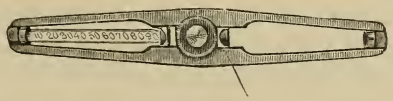


Fig. 15.—Daland Hematokrit, showing one percentage tube in position. (A. H. T. Co.)

necessary to multiply this by 4000 and this product by the dilution.

Example.—Suppose 1200 cells have been counted in 400 squares, then the average in one small square is three; this, multiplied by the number 4000, will equal 12,000, the number of corpuscles contained in 1 cubic millimeter of diluted blood, since the dilution was 200 times; then 12,000 x 200 will equal 2,400,000 red blood-cells in 1 cubic millimeter of undiluted blood.

Determination of the Erythrocytes by the Centrifuge.—The Daland hematokrit (Fig. 15) offers a quick, simple, and accurate method of determining the number of red corpuscles in the blood. The two great advantages of this process are, first, the elimination of the personal equation, and, second, as there is no dilution of the blood required, the frequent source of error arising from this cause does not enter in.

The hematokrit consists of an extremely light, though very strong, metal frame containing two glass tubes, each 50 milli-

meters long and graduated in one hundred parts, and having a uniform lumen of 0.5 millimeter.

The length of the percentage tubes and their distance from the center of the revolving hematokrit frame must always be the same as was used in the original experiments by which the comparative determinations were made, since any variations in these factors will produce corresponding variations in the results obtained.

The Technic.—The blood having been secured as outlined on page 49, the blunt end of the graduated percentage tube is attached to a suction pipette and the tube completely filled with freshly drawn blood. After any excess of blood has been carefully wiped away, the blunt end of the tube is placed in the distal cap of the hematokrit frame, and the other end pressed down upon the inclined plane of the proximal cap until it falls into and locks in the cavity. The second tube should be similarly treated and placed in the opposite side to serve as a check. It is absolutely necessary that the entire procedure should be performed with celerity so as to anticipate coagulation. After filling, immediately place the frame upon the spindle of the high-speed centrifuge, and turn the handle at the uniform rate of eighty turns per minute for exactly two minutes. This will produce 10,000 revolutions per minute in the spindle. At the expiration of this time all the corpuscles will be found to occupy the distal end of the tube; a thin, almost invisible, whitish line of white corpuscles appears between them and the plasma, which will be found to occupy the proximal portion of the tube. With the aid of a hand lens the height of the column of red corpuscles is read off, and the reading multiplied by 100,000, which converts the volume percentage into the number of corpuscles per cubic millimeter of the blood under examination.

The speed of the handle of the centrifuge must be uniform throughout the whole period, because this rate of rotation is the measure of a definite amount of centrifugal force generated in the revolving arms of the hematokrit frame. If the rate of rotation is either increased or decreased, the height of the column of cells will be similarly affected, thereby producing either too low or too high a reading.

A possible source of uncontrollable error is the variation

in size of the individual erythrocytes which occurs in certain pathologic states. The most accurate results by this method will, therefore, be obtained when the corpuscles are of uniform size. This fact has been taken advantage of in the estimation of the volumetric quotient, and its relation to diagnosis and prognosis in certain blood conditions (see below).

The Percentage of Erythrocytes.—To obtain the percentage of red blood-corpuscles, take the first two left-hand figures of the count in 1 cubic millimeter and multiply these by two; this will represent, approximately, the percentage of red blood-cells.

EXPLANATION.—If we consider normal blood to contain exactly 5,000,000 red corpuscles, then the figure 50 x 2 would equal 100 per cent., or normal. If, however, only 4,790,000 were counted in 1 cubic millimeter of blood, then 47 x 2 will equal 94 per cent. of erythrocytes.

The Color-Index.—From a knowledge of the percentage of hemoglobin and the percentage of cells, we are in a position to calculate the average color-index per cell, *i.e.*, its relative hemoglobin-content as compared with the normal cell.

EXAMPLE.—If the percentage of hemoglobin be 100 and the percentage of cells 100, then the color-index of each cell would be 1.0 or normal, viz.:—

$$\frac{\text{100 \% hemoglobin}}{\text{100 \% red cells}} = \text{color-index 1.0.}$$

If the percentage of hemoglobin be 67, and the percentage of corpuscles 90, then each individual cell will contain less than a normal amount of hemoglobin:—

$$\frac{\text{67 \% hemoglobin}}{\text{90 \% red cells}} = \text{color-index 0.74.}$$

The Volumetric Quotient or the Volume-Index of the Erythrocytes.—The investigations of Capps[2] have demonstrated that the normal volume obtained by the centrifuge hematokrit is 50 per cent. in the normal specimen. This volume he designates as 1. The volume in pathologic alterations can be cal-

[2] Jour. Med. Research, vol. x, 3, Boston, 1903.

culated in percentage of the normal volume, just as is the amount of hemoglobin estimated under similar circumstances. Having determined the volume by the hematokrit the erythrocytes are next counted and their number expressed in percentage by comparing with the normal. By dividing the volume of erythrocytes by the number of erythrocytes in the same blood (both expressed in percentage), we obtain the "volume-index" or "volume-value" of the erythrocytes, which is analogous to the color-index of hemoglobin as obtained above. This number in a measure of the average volume of the individual erythrocyte, and obviously under normal conditions, equals 1. Capps found that an increase in the volume-index of the erythrocytes is one of the most constant and accurate characteristics of pernicious anemia, which agrees with the well-known fact that many macrocytes appear in the blood in this disease, and that the color-index is also greater than one. In contrast to this, the so-called secondary anemias usually show a diminished volume-index; the same is true of chlorosis, in which affection the volume-index may be taken into account in determining the prognosis, since a normal or only slightly decreased volume-index gives a favorable prognosis, whereas a markedly diminished volume-index may be considered a bad prognostic sign.

When utilized in this way the volume-index may become a more reliable sign in chlorosis than the percentage of hemoglobin or the color-index.[3]

Estimation of the Leukocytes.—The Thoma-Zeiss apparatus is used for this estimation. The "white" pipette is of larger caliber than the "red" pipette, and makes a lower dilution (1 to 10 or 1 to 20). For diluting it is customary to use a 0.3 or 0.5 per cent. solution of acetic acid. This solution preserves the white cells, at the same time decolorizing the erythrocytes, thus facilitating enumeration. (For other diluting fluids see Appendix, page 404.)

For mixing and preparing the slide the same method is employed as described for the red cells, except that the 1 to 20 tube is employed. It is customary to count the total number of squares (400) (Fig. 16), and to multiply the total number of

[3] Sahli's "Diagnosis," quoting Capps: *loc. cit.*

cells counted by two hundred; the result will be the total number of white cells per cubic millimeter of blood.

ENUMERATION OF THE LEUKOCYTES.—*Explanatory note:* $\frac{1}{4000}$ cubic millimeter equals the cubic capacity of one small square. $\frac{1}{20}$ equals the dilution of the blood, and 400 (all) the small squares counted. Then the number of leukocytes per millimeter of undiluted blood will be found by dividing the number of cells counted by the number of squares counted and multiplying by the dilution times 4000, viz.:—Let x equal the number of leukocytes per cubic millimeter, and y equal the number of cells counted in 400 small squares. Then, $\frac{y}{400} \times 20 \times 4000 = x$ or, $y \times 200 = x =$ leukocytes per cubic millimeter of undiluted blood.

Improved Methods of Counting.—It is an advantage when counting the leukocytes to use a Zappert ruled disc. This has

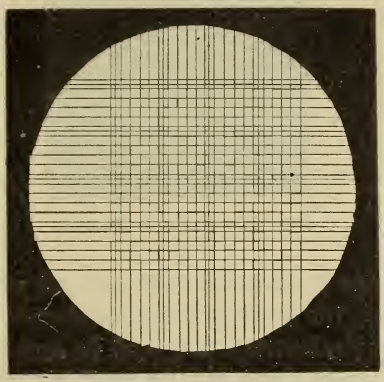


FIG. 16.—RULING OF CHAMBER OF THOMA-ZEISS HEMOCYTOMETER.

upon it the same ruling of 400 small squares in the central square millimeter as in the Thoma-Zeiss, but around it, in addition, are ruled eight other squares of the same size, which if required may each be divided into four. Each large square represents $\frac{1}{10}$ cubic millimeter when the counting chamber is covered.

When using Zappert's chamber, first the leukocytes in the central square millimeter, and then the other eight squares, are

counted with a low power, the normal being about 35 per square, with a dilution of 1 : 20. The formula would be:—

$$\frac{\text{Number of leukocytes counted} \times \text{Dilution} \times \text{Cubic contents of square}}{\text{Number of squares counted, 9}} = \left\{\begin{array}{l}\text{Number of leukocytes}\\ \text{per c.mm. (7133).}\end{array}\right.$$

$$\frac{321 \times 20 \times 10}{9} = 7133$$

This modification has been improved by Ewing and by Türck (Fig. 17) in such a way that the four large corner squares, each of 1 square millimeter, are subdivided into 16 small squares, each of which is equal in area to the total smallest 25 squares of the Thoma chamber.

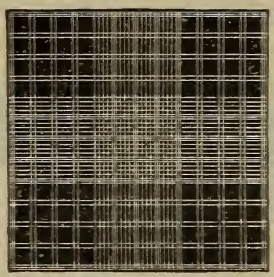


FIG. 17.—TÜRCK'S RULING.

Its advantage in counting both red and white cells will be appreciated when the student compares it with the older chamber. The 16 central squares are used in counting the erythrocytes, while the entire area may be used in the enumeration of the leukocytes. Simon has also introduced a different modification of the Thoma ruling.

CLEANING BLOOD TUBES AND PIPETTES.

Nitric acid is not best for cleaning the tubes, because it tends to form a coagulum within the tube which is hard to remove. A solution of citric acid followed by water, then alcohol and ether, is better. If any coagulum forms, caustic soda or caustic potash will remove it.

The Counting Diaphragm.—The process of counting may be simplified by using a diaphragm over the eyepiece,

which has a central square aperture, the area of which, with the same lens, eyepiece, and length of draw-tube, exactly corresponds to the area of the magnified 400 small squares, *i.e.*, $^1/_{10}$ cubic millimeter. With the covered chamber any area of it viewed through this diaphragm is equivalent to $^1/_{10}$ cubic millimeter, so that after being once adjusted any part of the field may be counted without taking any note of the ruled lines. A movable stage is a great convenience. These diaphragms may be obtained with the instrument, or easily made from a piece of cardboard or thin metal.

COUNTING THE BLOOD-PLATELETS.

The technic of counting these cellular elements has, until recently, been imperfect and the results variable. Three years ago Wright and Kinnicutt[4] introduced a method which seemed simple, exact, and fairly reliable. The technic is as follows: The blood is diluted 1:1000 by means of the pipette used for counting the red cells, and the counting is done in the Thoma-Zeiss counting chamber, using all the precautions previously mentioned. The specially thin cover-glass of Zeiss, with central excavation, is used to render the platelets clearly visible. The diluting fluid consists of 2 parts of a 1:300 aqueous solution of "brilliant cresyl blue" and 3 parts of a 1:400 aqueous solution of potassium cyanid. These two solutions should be fairly fresh, kept separate, to be mixed and filtered just before taking the blood. After the counting-chamber has been filled, ten to fifteen minutes should elapse before counting, in order to allow the blood-platelets to settle to the bottom of the chamber and so be more easily and accurately counted. The platelets appear as sharply outlined, round, oval, or elongated, lilac-colored bodies, some of which form a part of small spheres or globules of hyaline substance. The red cells are decolorized and appear as "shadows," while the nuclei of the leukocytes are stained a dark blue and their protoplasm light blue. This method shows a normal platelet count of 225,000 to 350,000 per cubic millimeter. No constant relations seem to obtain between the variations in the numbers of platelets and of the leukocytes. Accord-

[4] Jour. Amer. Med. Assoc., vol. lvi, 1911, p. 1457.

ing to Determann, the ratio between the red cells and the blood-platelets is, on the average, 22 : 1.

MICROSCOPIC EXAMINATION.

Examination of a Drop of Fresh Blood.—If a drop of fresh blood is placed upon a slide and a perfectly clean cover-glass allowed to fall upon this, a fresh preparation for examination will be produced, which may either be examined immediately or, if sealed around the edge with a little vaseline, may be carried to the laboratory and examined within an hour. With the aid of a 6 objective many interesting points may be observed. First, the shape of the *red cells* and their hemoglobin staining; also rouleau formation. Second, the structure of the *white corpuscles* by which the different varieties may be roughly differentiated. Third, the *blood-platelets*. Fourth, a rough idea of the relative number of red and white cells may be formed. This matter will be discussed more in detail below.

Studies of the minute structure of the leukocytes cannot be made satisfactorily by this simple method, as some of the forms are present only in small numbers, and are detected with difficulty. Further, prolonged examination of the fresh specimen allows time for changes, which rapidly obscure the identity of the cells; hence we are obliged to resort to a method of preparing a specimen which shall be permanent and which can be stained as a further aid to differentiation. These methods have the added advantage that they allow the investigator to work quite independently of the presence of the patient, to choose the time and place of examination, and to permit at any time of verification and demonstration of the original result obtained.

Preparation of the Specimen.—The blood is obtained from the tip of the finger or the lobe of the ear after the manner described above. Cover-glasses should previously be prepared for reception of the fresh blood as soon as it is drawn. The cover-glasses should be square, seven-eighths or one inch in diameter, and of special thinness (0.1 to 0.08 millimeter). They should be washed carefully with warm water and soap, dried with fat-free gauze, and finally wiped off with ether. This

is necessary to insure proper spreading of the blood between the two glass surfaces, which would be prevented by traces of fat or particles of fiber, dust, etc. Just before the glasses are used they should be wiped off with a piece of silk or tissue paper, and thereafter handled with care by the corners, or, better still, with forceps. This preparation of the cover-glasses may be done in the laboratory or carried on at the bedside, as desired.

The glasses being ready and the finger cleansed and punctured, a small drop of blood is allowed to exude without pressure. Now a cover-glass is taken up diagonally between the thumb and finger of one hand, and its center allowed to touch the drop of blood. Immediately taking another cover-glass in the same manner in the other hand, it is allowed to fall upon the drop. If the glasses are perfectly clean and the maneuver properly executed, the blood will immediately spread out in a uniform layer between the two glasses. The two cover-glasses are now separated one from the other by a rapid sliding motion, in the strict plane of their surfaces, without the slightest lifting or tilting.

If the drop taken was small enough and the technic properly carried out in every detail, the result will be two uniform smears, covering the larger portion of the surfaces of the cover-glasses, which, when examined under the microscope, will present a uniform layer of corpuscles, with little if any overlapping of the individual cells.

Usually six films are prepared in this manner and allowed to dry in the air without heat.

Methods of Fixation.—In using the Romanowski stains no previous fixation of the albuminous film is required. Before applying any of the other stains it is necessary to "fix" the blood-film. If this is not done the corpuscles will be washed from the glass by the fluids applied.

1. A strip of sheet copper eighteen inches long and two or three inches wide is placed upon a stand, and the flame from a Bunsen burner placed under one end. After allowing sufficient time for the plate to attain a maximum heat the films are placed upon it at a point where a drop of cold water fails to roll off, but adheres to the hot metal and steams away. At

this point fixation is complete in from one-half to three-quarters of an hour.

2. Fixation may be accomplished by immersion for five to ten minutes in a mixture of 1 part formaldehyde solution and 99 parts absolute alcohol.

3. For routine work when fair accuracy, accompanied by speed in obtaining results, is necessary, fixation in the naked flame of a Bunsen burner may be practised. This is accomplished by holding the dried smear diagonally between the thumb and forefinger of one hand, film side up, and passing it through the flame a number (five to ten) of times with sufficient rapidity to prevent the fingers being burned.

Methods of Staining: *Eosin and Methylene-blue.*—The fixed film held in the cover-glass forceps is flooded with a ½ per cent. solution of eosin in 50 per cent alcohol, which is gently washed off with distilled water after the lapse of one or two minutes. The counter-stain of methylene-blue, 1 per cent. aqueous solution, is then added and allowed to act for from two to five minutes, according to the density of the film. Finally, washing in distilled water, blotting and drying, complete the process prior to mounting and examination.

This is an extremely simple, yet effective and permanent, method of staining blood-smears. Little difficulty will be experienced in obtaining satisfactory specimens. The eosin should not be allowed to act too long, but there is little danger of overstaining with the methylene-blue. Both of these stains are permanent when prepared, and can be kept until exhausted.

Properly performed, this method gives very vivid and contrasting pictures, the nuclei of the different white cells taking various shades of blue, pale in the lymphocytes, and dark in the polymorphonuclears. The protoplasm appears faintly tinted blue (paler than the nuclei). The eosinophilic granules are a bright pink; the erythrocytes a dusky red.

Ehrlich's Triacid Staining Method (for formula and preparation see Appendix).—The smear is taken in the cover-glass forceps and a few drops of the prepared stain placed upon it and allowed to remain for about five minutes. There is little danger of overstaining. The specimen is next washed in distilled water and thoroughly dried prior to mounting. If

PLATE II



NORMAL AND PATHOLOGICAL BLOOD-CELLS.

1, Normal red cell, or erythrocyte; diameter, 7.5 μ. *2*, Nucleated red cell, or normoblast; diameter, 7.5 μ. *3*, Megaloblast; diameter, 8 to 12 μ; seen in pernicious anemia. *4*, Small lymphocyte; diameter, 6 to 8 μ; average in normal blood, 20 to 26%. *5*, Large lymphocyte; diameter, 8 to 13 μ; average in normal blood, 5 to 9 %. *6*, Polymorphonuclear leukocyte; diameter, 10 to 11 μ; average in normal blood, 65 to 70%. *7*, Eosinophile; diameter, 19 to 12 μ; average in normal blood, ½ to 2%. 8, Large mononuclear leukocyte; diameter, 12 to 18 μ; average in normal blood, 1 to 2%. *9*, Transitional; diameter, 12 to 17 μ; average in normal blood, 2 to 3%. *10*, Neutrophilic myelocyte; diameter, 12 to 20 μ; seen in myelogenous leukemia. *11*, Eosinophilic myelocyte; diameter, 10 to 16 μ; seen in myelogenous leukemia.

mounted in Canada balsam it is imperative that this should not contain any chloroform; otherwise the colors will gradually become blurred.

The Romanowski or "Universal" Staining Method.—Leishmann's modification of the Romanowski stain, as made by Wright[5] (see Appendix for stain), is the stain used. This method is altogether quicker and easier than the method of Ehrlich. It requires no fixing fluid or heating apparatus, and gives pictures which are uniformly superior.

Technic.—Allow three or four drops of the prepared stain to fall upon the smear and permit it to remain one-half minute, rocking the cover gently so as to insure an even distribution of the stain. No attempt is made to check evaporation. At the end of one-half minute add double the quantity of distilled water, *i.e.*, six to eight drops, and allow it to mix with the alcoholic stain. Immediate mixing is hastened by gently rocking the cover-glass. The film is now allowed to stain for five minutes. In thick smears ten minutes may be necessary. The stain is now gently washed off with distilled water, and a few drops of water are allowed to rest on the film for one minute, when it should be dried in air and mounted.

Appearance of Stained Blood-films.—Erythrocytes, pale pink or greenish, semi-transparent.

Polymorphonuclears.—Nuclear network, stained a very ruby-red color, or with sharply defined margins. Extra-nuclear protoplasm, colorless. Fine eosinophilic granules, red.

Mononuclears.—Nuclei ruby-red with extremely sharp, clear outlines. Extra-nuclear protoplasm, pale eau-de-nid or blue, occasionally showing a few red granules.

Lymphocytes.—Same as mononuclears, except that as a rule the nuclei are more deeply stained.

Coarse-grained Eosinophiles.—Nucleus only, red, but not so densely stained. Granules pale pink.

Basophiles.—Granules very densely stained of a very purplish black tint. Nucleus red, but usually more or less meshed by granules overlaying it.

Nucleated Red Cells.—Nucleus almost black, with sharp outline, extra-nuclear portion gray.

[5] Wright: Jour. Med. Research, vol. vii, 1902.

Blood-plates.—Deep ruby-red with spiky margins, frequently showing a pale-blue spherical zone surrounding the red center.

Bacilli and Micrococci.—Speaking generally, these stain evenly blue, but by prolonging the staining period and subsequently decolorizing with absolute alcohol many interesting variations may be noted with different organisms by which structural details are brought out not generally observed by other staining methods.

Malarial Parasites.—The body of the parasite stains blue and its chromatin ruby-red. In the case of the tertian parasite, Schuffner's dots are well marked in the containing red blood-corpuscles.

The only weak points in the Romanowski stain are said to be the deceptive resemblance between megaloblasts, certain lymphocytes and certain myelocytes, and failure to differentiate the basophiles.

Eosin-hematoxylin.—This stain is especially important in cases in which the nuclear structures are to be studied. It stains the nuclei beautifully, showing their fine structure, karyokinetic figures, and pycnotic qualities, as well as the basophile granules of both red and white cells (for preparation of reagents see Appendix, page 408).

Technic.—Stain the specimen with the eosin solution for one-half minute, and wash in water. Without drying place the slide in the hematoxylin solution for one to three minutes, the time varying with the particular stain and with the experience of the worker. Wash with water, dry, and mount.

TERMS IN COMMON USE IN CLINICAL HEMATOLOGY.

Anemia.—A condition of the blood in which there is a deficiency in one or more of the normal constituents.

Anhydremia.—A deficiency in the normal fluid of the blood.

Basophilic Granulation.—A peculiar granular degeneration of the red blood-cells which is noted in chronic lead-poisoning.

Hydremia.—An excess of fluid in the blood.

Leukocytosis.—An increase above the normal number of white blood-cells.

Leukopenia.—A diminution in the number of white blood-cells.

Lipemia.—The presence of an abnormal amount of free fat in the blood.

Macroblasts.—Nucleated red blood-cells of more than normal diameter.

Macrocytes.—Abnormally large red blood-cells.

Melanemia.—The presence of free pigment in the blood.

Microblasts.—Nucleated red blood-cells of abnormally small size.

Microcytes.—Abnormally small red blood-cells.

Normoblasts.—Nucleated red blood-cells of normal diameter.

Oligemia.—By this term is meant a reduction in the total volume of blood, both as regards the liquid and the cellular portions.

Oligochromemia.—A diminution in the normal amount of hemoglobin. This may occur either independently or coincidently with a diminution in the number of red blood-cells.

Oligocythemia.—A diminution in the number of red blood-cells.

Plethora.—An increase in the total quantity of blood above normal.

Poikilocytosis.—This term is applied to the very irregular shape of the erythrocytes observed in certain pathologic conditions.

Polychromatophilic Degeneration or Anemic Degeneration (Ehrlich).—This is an atypical staining reaction of the erythrocytes, the significance of which is not yet definitely determined.

Polycythemia.—An increase in the number of red blood-cells as compared with the fluid-content.

VARIETIES OF LEUKOCYTES.

1. **Normal Leukocytes.**—(a) Lymphocytes: These are derived from the lymph-glands, and appear as small, round cells about the size of a red blood-corpuscle, with a large, centrally located nucleus, and a small margin of protoplasm. The nucleus stains rather intensely with the nuclear stains (hematoxylin, methylene-blue, and Ehrlich's triple stain). The protoplasm is free from granules.

(b) Large Mononuclear Leukocytes.—These cells are two or three times as large as a red blood-cell, with a large, usually oval nucleus which is generally eccentrically placed. They stain poorly with nuclear stains. There is a relatively large amount of protoplasm, which is free from granules. They are derived from the bone-marrow, and may be regarded as the parent type of the polymorphonuclear.

(c) Polynuclear or Polymorphonuclear (Neutrophilic) Leukocytes.—These are recognized by their multiple, irregular-shaped or bent nucleus. The nuclei stain very intensely, and the protoplasm is densely packed with neutrophilic granules. (See page 75 for Arneth's classification.)

(d) Eosinophilic Cells.—These resemble the polymorphonuclear cells, except that the small neutrophilic granules are replaced by coarse acidophilic (eosinophilic) granules. These granules are highly refractive, so that they can be readily recognized without staining.

(e) Mast Cells.—These are cells of the polymorphonuclear type with marked basophilic granules, which are quite large, uneven, and irregularly distributed. They are not distinctly stained by the triple stain.

2. **Pathologic Leukocytes.**—(a) Mononuclear Neutrophilic Cells (Myelocytes): These are large cells with a large, faintly staining nucleus, differing from the large mononuclear cells of normal blood by the presence of neutrophilic granules in the protoplasm.

(b) Mononuclear Eosinophilic Cells (Ehrlich's Eosinophilic Myelocytes): These cells are what their name implies, mononuclear eosinophiles. Very small cells of this type have been termed *eosinophilic microcytes*.

THE DIFFERENTIAL COUNT.

The distinguishing characteristics of the various white cells, as brought out by any one of the differential stains above outlined, allow the investigator to separate these into several groups, and thus to estimate their relative numbers, which are usually expressed in percentage. The cells for a differential count can be enumerated without the aid of a mechanical stage, but this instrument of precision is a distinct aid in the per-

formance of this procedure and should always be used when possible. Select a uniform and not too dense part of a stained smear, and adjust upon the mechanical stage so that the field presents cells in uniform arrangement without any overlapping. Now, by means of the thumb-screws of the mechanical stage, the field is carried back and forth before the eye of the observer, so that the same part of the field is not brought into view more than once (this matter is very simple when a mechanical stage is used). As the parade of cells passes before the eye, the white cells are observed, classified, and the number jotted down. This is continued until not less than two hundred (see page 74 for computing chart to facilitate the count), and preferably five hundred or a thousand, cells have been counted. With the total number of cells counted known, and also the number of cells in each class recorded, it is a simple matter to calculate the percentage of each variety of cell in the specimen examined.

A Computing Chart for the Differential Leukocyte Count.—The accompanying chart (Fig. 18) has been suggested by Dr. Osmond[6] to simplify the work and remove the sources of error in the differential count. The chart is inexpensive and can be made out of a piece of ground glass or of slate suitably ruled and marked in ink. The temporary markings are made in pencil and can be readily erased. It is figured out on a basis of two hundred cells and the marks on the left indicate the various types of cells encountered. At the bottom are the calculated percentages and at the top the actual number of cells counted when the vertical columns are filled.

The heavy lines running vertically facilitate the count by indicating when fifty, one hundred or one hundred and fifty units are counted for a certain type of cell. By referring to the figures in the top row one can readily read the actual number of cells of each type counted and easily sum up when the total of two hundred has been reached. This being done, the percentages can be read off directly from the bottom figures, no calculation to determine these being required.

The Normal Differential Count.—The normal leukocytic count may vary between 5000 and 10,000 white cells per cubic

[6] A. E. Osmond: Jour. Amer. Med., Jan. 8, 1910.

millimeter. The average normal leukocyte count is usually placed at 7500 leukocytes per cubic millimeter.

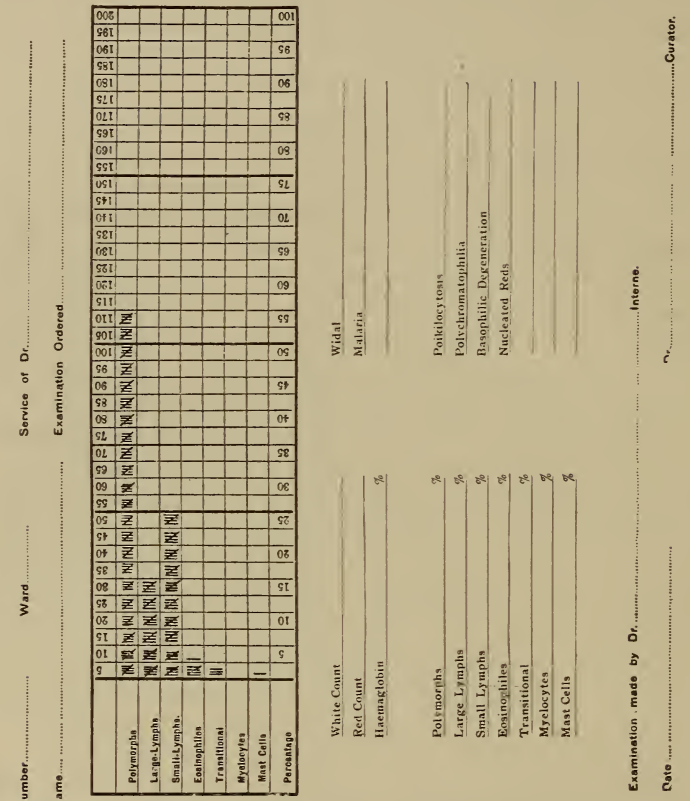


FIG. 18.—A COMPUTING CHART TO FACILITATE MAKING A DIFFERENTIAL LEUKOCYTE ESTIMATION. (DEVISED BY DR. A. E. OSMOND.)

The minimum and maximum number of cells for each type, estimated from the minimum normal (5000) leukocyte count, is given in the following table:—

VARIETY.	MINIMUM.	MAXIMUM.
Polymorphonuclears	3000	3500
Small lymphocytes	1100	1500
Large lymphocytes	250	350
Eosinophiles	50	100
Mast cells	5	25

The minimum and maximum number of cells of each cell-type estimated from the maximum normal leukocyte count (10,000) :—

VARIETY.	MINIMUM.	MAXIMUM.
Polymorphonuclears	6000	8000
Small lymphocytes	2200	3000
Large lymphocytes	500	900
Eosinophiles	100	200
Mast cells	10	50

For the average normal standard for each cell-type we may adopt the following standard:—

VARIETY.	NUMBER.	PER CENT.
Polymorphonuclears	4875	65
Small lymphocytes	1950	26
Large lymphocytes	525	7
Eosinophiles	75	1
Mast cells	8	0.1

ARNETH'S CLASSIFICATION.

Arneth,[7] in his study of the blood both in health and disease, has been led to classify the polymorphonuclear neutrophiles into five classes, depending upon the number of nuclear lobes. His divisions seem to be fairly constant in health, but show great variation, especially in infectious conditions. Reduced to tabular form, the Arneth classification appears on the following page.

Clinical Significance.—It is probable that these various classes represent the gradual development of the polymorphonuclear neutrophile; the older the cells, the greater the tendency to reach Class 5, while in conditions associated with new and rapid cell formation, as in infectious conditions, we find the percentage of the earlier classes being increased, that of the later ones diminished.

L. H. Briggs,[8] in his investigation of the several types of neutrophile, found that the nuclear formula in the normal agrees closely and constantly with that found by Arneth and many other workers. In tuberculous, typhoid, pyogenic, and

[7] T. Arneth: The Neutrophile Blood-picture in Infectious Diseases, Zeit. f. klin. Med., lxvi, No. 2, 1908.

[8] California State Journal of Medicine, August, 1912, x, No. 8.

TABLE OF ARNETH'S CLASSIFICATION OF LEUKOCYTES.[9]

Class	Subclass A	B	C	D	E	Occurrence by class
I Single nucleus	M-Cells Ehrlich's myelocytes	W-Cells Slightly indented nucleus Meta-myelocytes	T-Cells One deeply indented nucleus			I M-Cells, always abnormal. W-Cells, 0.2% T-Cells, 5.0%
II Two nuclear divisions	2 K-Cells Two round nuclear portions	2 S-Cells Two distinct S-shaped forms 23%	1 K-1 S-Cells One round, one S-shaped nuclear portion			II 35%
III Three nuclear divisions	3 K-Cells	3 S-Cells	2 K-1 S-Cells 16%	2 S-1 K-Cells 16%		III 41%
IV Four nuclear divisions	4 K-Cells	4 S-Cells	3 K-1 S-Cells	3 S-1 K-Cells	2 K-2 S-Cells	IV 17%
V	Five nuclear subdivisions, which may be arranged into classes, but this is unnecessary, owing to their very small percentage.					V 2%

[9] Table arranged by author.

malarial infections a deviation from this normal is regularly found, the so-called "shift to the left," consisting of an increase in the cells with fewer nuclear units and a corresponding decrease in the cells with many. The degree of this shift appears to be roughly proportioned to the severity of the infection, except in typhoid, in which it is uniformly present to a marked extent, irrespective of the individual case. In tuberculosis such changes in the neutrophiles seem to offer a distinct aid in prognosis, although its reliability needs further confirmation.

A Simple Method of Counting Eosinophile Leukocytes.—Dunger [10] dilutes the blood in the proportion of 1:10 with the following solution: 1 per cent. aqueous solution of eosin and acetone, of each, 10 cubic centimeters; distilled water, 100 cubic centimeters. The eosinophiles can then be seen in a bright rose field as roundish spheres composed of shining red granules that can be easily recognized even by the inexperienced. They can then be counted much more readily than after preparations in the older ways. He considers that the eosinophiles form a very sensitive indicator in the various infectious diseases and furnish a valuable aid in prognosis.

LEUKOCYTOSIS.

For convenience in the study of the various forms of leukocytosis, they may be divided into two classes: (*a*) Physiologic leukocytosis. (*b*) Pathologic leukocytosis.

(a) **Physiologic Leukocytosis.**—The average number of leukocytes, per millimeter of blood, is normally 7500. For children the average is slightly more. For weak and poorly nourished persons slightly less. The numbers of leukocytes in the peripheral blood of any individual vary from time to time. They (1) may be increased after a hearty meal, especially if it contains much proteid material. Physiologic leukocytosis may also occur (2) during pregnancy, particularly during the latter months of the condition; (3) in the new-born, and (4) after cold baths.

In these so-called physiologic leukocytoses the increase does not usually exceed 30 per cent. of the normal, though in children it may be doubled.

[10] Münch. med. Woch., September 13, 1910.

A *hypo-leukocytosis* is said not infrequently to precede a hyper-leukocytosis.

(b) **Pathologic Leukocytosis.**—Many infections cause an increase in the number of white corpuscles in the peripheral circulation. Although the varieties of cells are the same as in health, the relative proportions are usually altered. In the more common forms of pathologic leukocytosis, which is seen in the common infections, the percentage of lymphocytes is diminished, while the polymorphonuclears are frequently increased from normal (65 per cent.) to 90 or 95 per cent.

A *polymorphonuclear leukocytosis* occurs especially during inflammatory processes, and above all in those accompanied by purulent exudation.

In certain infectious diseases, notably typhoid fever and uncomplicated tuberculosis, there is usually no increase in the number of white blood-cells.

The origin of the extra leukocytes has not yet been definitely determined, as we do not know whether they are derived from the bone-marrow, lymph-glands, or from other tissues.

Another form of leukocytosis is characterized by a relative increase in the number of *eosinophilic* leukocytes. This condition of the blood is notably observed in bronchial asthma, trichinosis, and infections with other animal parasites, and has lately been reported in Hodgkin's disease. It is of interest to note that in these conditions there usually exist local collections of eosinophiles at the seat of disease. These accumulate in the walls of the bronchi and in the exudate in bronchial asthma, and about the embryos in trichinosis.

The number of white cells in a pathologic leukocytosis not infrequently reaches 20,000 to 30,000 cells per cubic millimeter, and has been known to reach the enormous number of 168,000 (Grawitz).

Leukopenia.—A diminution in the number of leukocytes in the peripheral blood occurs in a variety of conditions. It has been observed in cachexias, in intoxications, many anemias, and in some infectious diseases, notably in typhoid fever and in malaria.

In leukopenia, as in leukocytosis, the relative proportions of the various varieties of white cells are usually changed. For

example, in typhoid fever there is often a relative increase in the number of lymphocytes.

THE ANEMIAS

Anemias are conveniently classified as **primary** when due to some unknown cause, and in which the blood-changes are, as a rule, of both a quantitative and a qualitative type.

And as **secondary,** when the cause is known and when the blood-changes are usually of a quantitative type only.

The Secondary Anemias.—Causes: Secondary anemia is observed after hemorrhage, during pregnancy, during chronic and constitutional diseases, and in poisoning, including that large and vague group of conditions comprising auto-intoxication. In chronic digestive disorders, malignant tumors, tuberculosis, syphilis, malaria, and in the different forms of helminthiasis.

The Blood-changes.—The chief blood-changes in secondary anemia consist in a reduction of hemoglobin and a diminution in the number of red blood-cells. Mild forms show no other changes in the blood-picture. Severe forms show poikilocytosis, macrocytosis, and microcytosis. The extent to which these conditions are observed corresponds roughly to the severity of the anemia.

Further, the red corpuscles often manifest a change in reaction to the ordinary staining reagents. They stain poorly, unevenly, and some parts of some cells refuse entirely to take the stain. This condition is termed polychromatophilia. This change is no indication of the grade of the anemia, as it may be observed in the mildest forms of secondary anemia.

The white cells in the simple anemias present nothing that is characteristic.

Summary.—The essential blood-changes in the secondary, or simple anemias consist in a diminution in the hemoglobin percentage, and in the number of red blood-cells. The red cells may show polychromatophilic degeneration and poikilocytosis.

The Primary Anemias.—General Considerations: Unlike the secondary anemias, the blood-changes in primary anemias, besides showing any or all of the modifications observed

in the former, present very striking and characteristic alterations in the white blood-cells.

Progressive Pernicious Anemia.—In contrast to the process in simple anemia, in progressive pernicious anemia, blood-degeneration in certain portions of the blood-making organs, notably in the bone-marrow, takes place in a manner different from the physiologic. Consequently, in the blood-formative organs and also in the circulation, we note cells often in great numbers that are never seen in the normal blood. These pathologic elements are present in embryonal life; so in pernicious anemia we speak of the reversion of blood-formation to the embryonal type.

The Blood-changes.—In a typical, well-defined case of pernicious anemia, the first glance at a well-prepared stained specimen of blood is sufficient to separate it immediately from the class of simple anemias. We find that a large number of erythrocytes have a diameter greater than normal (megalocytes: diameter 15 to 16 microns). These cells by their staining show a great richness in hemoglobin. Careful search always shows some megaloblasts, *i.e.*, nucleated (embryonal) red corpuscles. Normoblasts and microcytes are also in evidence. Other changes usually present are poikilocytosis, polychromatophilia, and granular degeneration.

The red corpuscles are always notably decreased, and may be less than 20 per cent. of the normal. The percentage of hemoglobin is also diminished, but practically always to a less degree than the red cells. On account of this condition the color-index is very frequently above 1.0.

CHLOROSIS.

Blood-changes.—The blood when drawn flows freely from the puncture, and is markedly watery. Hemoglobin estimation shows a decided reduction in the percentage of hemoglobin without a corresponding deficiency in the number of red blood-cells. The color-index is therefore low, below 1.0, in contrast to the high color-index observed in pernicious anemia. This particular characteristic is not unique in chlorosis, as other forms of anemia may also show it.

Morphologically we find striking changes in the erythrocytes (many appear as macrocytes), which are pale and are

without a distinctly pronounced central umbilication—the cells appear swollen. These particular cells have been designated "chlorotic" blood-corpuscles. Severe cases show poikilocytosis and nucleated red blood-cells.

Polychromatophilia and granular degeneration, which are the genuine phenomena of degeneration, are not observed.

The condition of the leukocytes is not uniform. The cells themselves do not show any characteristic changes in this disease.

The blood-plaques appear in markedly increased numbers, so that many groups of these cells appear in every field of the fresh preparation.[11] These are often very evident in the stained preparation, especially if a basic stain, such as methylene-blue, has been used.

LEUKEMIA.

Changes in the Number of Erythrocytes.—The blood from a marked case of leukemia is distinctly watery; in extreme cases it may be a whitish-red as it emerges from the puncture; this is owing to the great increase in white elements.

The microscopic examination of the fresh blood in established cases shows, even without counting, the enormous increase in the white blood-cells. The count of the white corpuscles shows 100,000 to 500,000, or even more, to the cubic millimeter.

In some cases of leukemia no noteworthy change in the erythrocytes, either in number or appearance, occurs. As a rule they are decreased to about half the normal number. Besides the diminution in these cells the blood contains varying numbers of normoblasts, and more rarely megaloblasts.

The amount of hemoglobin is diminished, but the coloring of the individual corpuscles need not be diminished (no alteration in the color-index).

Changes in the Blood-plaques.—An increase in the number of the blood-plaques has been noted in a number of cases.

Blood Morphology.—The diagnosis of leukemia can frequently be made without further consideration in cases which show an extraordinary increase in the number of white elements, but in doubtful cases the presence of the condition can only be

[11] E. Grawitz: Modern Clin. Med., 1906, p. 327, D. Appleton & Co.

determined by an exact examination of the morphology of the leukocytes.

Besides noting the great increase in the number of white cells, the polymorphonuclear elements will usually be found the most numerous.[12] In other cases the increase is chiefly among the lymphocytes. We may therefore differentiate two forms of leukemia.

1. Lymphatic Leukemia. 2. Leukocytic Leukemia.

Since the source of the lymphocytes is from the spleen and from the lymph-glands, and that of the leukocytes from the bone-marrow, the common designation of lymphatic leukemia (lymphemia) and myelogenous leukemia (myelemia) may be applied to these two basic forms of leukemia.

As, however, the bone-marrow normally produces typical lymphocytes, and as it has been demonstrated in rare conditions that an overflooding of the blood with lymphocytes may occur through proliferative changes in the bone-marrow without enlargement of the spleen or lymph-glands (myelogenous lymphemia), it seems preferable to apply the less prejudicial division of the leukocyte forms into "lymphatic leukemia" and "leukocytic leukemia" (W. von Leube).

Differentiation.—1. Lymphatic Leukemia: The blood-picture is conspicuous for its great preponderance of large and small lymphocytes, in comparison to the leukocytes. Nucleated red cells (normoblasts) and megaloblasts, although present in this form of leukemia, are by no means as abundant as in the leukocytic form.

2. Leukocytic Leukemia.—This is by far the most common form of leukemia. It may easily be differentiated from the preceding by the entirely different blood-picture. The increase in the white elements is usually very marked. Here, however, the polymorphonuclear leukocytes are greatly increased in the microscopic blood-picture. Neutrophiles and eosinophiles are absolutely always increased (Ehrlich). There is also an increase in the mast cells, which may be twice as numerous as the eosinophiles. Their determination is of the greatest importance, since a marked increase in mast cells is observed only in this disease (von Leube).

[12] W. von Leube: Modern Clin. Med., 1906, p. 349.

The phenomena which especially characterize leukocytic leukemia, and which result from changes in the bone-marrow (myelogenous leukemia), are the occurrence of neutrophilic and eosinophilic myelocytes. These may be present in enormous numbers (up to 100,000 per cubic millimeter). The first sight of such a picture simulates the blood-picture of acute lymphemia with its large mononuclear cells.

The *myelocyte* is a large mononuclear cell with an irregular nucleus, surrounded by a considerable amount of protoplasm, in which are either neutrophilic or eosinophilic granulations. Besides these immature leukocytes (myelocytes), immature forms of erythrocytes, also originating in the bone-marrow, are found in the circulating blood-stream of patients suffering from leukocytic leukemia. There are also normoblasts and occasionally megaloblasts.

HODGKIN'S DISEASE (PSEUDOLEUKEMIA).

General Lymphadenoma.—This is a variety of anemia characterized chiefly by progressive enlargement of the superficial lymph-glands. The blood-picture is that of a secondary anemia plus the leukocytosis occurring during fever periods, and often a leukopenia during the intermissions. The blood-picture in this disease varies with the stage. During the early part the hemoglobin is slightly reduced, and the red cells fall to from 3,500,000 to 2,000,000. There may be a slight increase in the number of leukocytes, but this leukocytosis rarely amounts to more than 18,000. In the later stages there may be a great reduction in both hemoglobin and red cells.

The differential count shows an increase chiefly in the polymorphonuclear element and an occasional myelocyte and some eosinophilia. According to Boston, in cases when the leukocyte count is normal, there is an increase in the percentage of lymphocytes.

DEGENERATED RED CELLS, RING BODIES, ETC.

Occasionally one observes in the red cells curious ring-like bodies which are very much like the hyaline malarial ring, with a circular refractive center. They change their shape in a peculiar way, much resembling the undulatory movements of

the hyaline body. They do not increase in number or grow larger on standing. They are observed in a large number of conditions, such as measles, pernicious anemia, and severe secondary anemias. Herrick [13] has observed thin, elongated, sickle-shaped red cells, some of them nucleated, in a severe anemia.

Various other forms of degeneration of the red cells occur, as, for instance, the appearance of rod-like areas resembling bacilli, which may keep up a constant vibratory motion carrying them through the entire substance of the cell. This finding should not confuse one in making a diagnosis of the presence of bacteria.

Another form of degeneration, known as Ehrlich's hemoglobinemic degeneration, has the appearance of a small dark cell lying upon a larger, paler one. These are probably areas of condensed protoplasm in which the hemoglobin is distinctly separated from the stroma. Such cells appear, occasionally, in certain types of malaria and are shown as corpuscles in which the hemoglobin is apparently condensed around the parasite. This degeneration is occasionally seen in nucleated red cells and may give the appearance of a microblast lying upon a macrocyte. It is best seen in cases of pernicious anemia and may explain some of the "acidophilic granules" of the red cells which have been described (Emerson).

VITAL STAINING.

Although the examination of stained specimens of the blood is usually made with the dried and fixed smear, excellent results may be obtained when fresh blood is stained without previous fixation. It is true that a "vital" staining of the blood-cells does not actually take place, as the dyes are decolorized by the reducing and oxidizing action of the living cells. However, a "postvital" staining—that is, the staining of whole cell or portions of the cells, after their removal from the circulation and before the death of the cell results—may be accomplished in several ways.

We may either add to the fresh drop of blood a very few

[13] Arch. of Int. Med., vol. vi, 1910, p. 175.

crystals of the stain, as advised by Arnold, and note the staining of certain leucocytic granules and nuclei and the reticular structure of many erythrocytes, or we may first dry a staining solution upon the slide, cover this dry stain with a drop of fresh blood, adjust the cover-glass, and seal this to the slide with paraffin or vaselin.

The stains which may be used for vital staining of the nuclei, granules, and plates are methylene-blue, toluidin-blue, thionin, neutral violet, Capri blue, Nile blue, brilliant cresyl blue, Janus green, and paraphenyl blue. Of the protoplasmic stains we have fuchsin, acridin red, pyronin, safranin, and neutral red.[14]

It may be possible by this method to differentiate between certain forms of degeneration of the cell which are now known only indefinitely under the names of metachromatic and polychromatic staining (see above).

Iodophilia.—The employment of a reagent containing iodine is used to demonstrate the presence of glycogen, which, when present in the white blood-cells, particularly the polymorphonuclears, is supposed to indicate the presence of a suppurative condition. Blood smears are made on slides or cover-glasses in the usual manner, and after they are dried, but without fixation, mount them in a drop of the following solution:—

Iodine	1 part,
Potassium iodide	3 parts,
Gum arabic	50 parts,
Water	100 parts.

The presence of small, brown masses in the polymorphonuclears or lying free indicates a positive iodophilia.

Significance.—These granules have been found by Hofbauer in pernicious anemia, secondary anemia, and leukemia, but not in chlorosis or pseudoleukemia. This reaction is observed in pneumonia, but is seldom seen in tuberculosis, typhoid fever, and diphtheria. It is, however, not to be regarded as dependent upon infection, as it occasionally obtains in non-infectious conditions.

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[14] Webster's "Diagnostic Methods."

Simple Test for Bile in the Blood.—A. Sunde[15] has found that it is possible to estimate the intensity of the admixture of bile with the blood with sufficient accuracy for all practical purposes by the length of time necessary for the color reaction to occur when the serum is treated with nitric acid. He used Scheele's modification of Gilbert's test, adding to 20 or 30 drops of blood-serum, in a test-tube not over 10 millimeters in diameter, a little of the reagent made by combining 300 parts nitric acid with 0.06 part sodium nitrite. The reagent is allowed to flow down the side of the tube, and a bluish ring forms at the junction of the two fluids. The interval before the bluish ring becomes evident varies from half a minute to thirty minutes when there is considerable bile in the blood, while with only small proportions the interval ranges from forty-five to sixty-five minutes or longer. It is possible thus to distinguish the pathologic from the non-pathologic cases; the shorter the interval, the greater the probability of some local affection in the liver or bile passages interfering with the normal evacuation of the bile.

SPECTROSCOPIC EXAMINATION.

Solutions of hemoglobin and its derivative compounds, when examined with the spectroscope, give distinctive absorption bands.

The Spectroscope.—Light, when made to pass through a glass prism, is broken up into its component rays, giving the play of rainbow colors known as the spectrum. A spectroscope is an apparatus for producing and observing the spectrum. In brief, the apparatus consists of a base or stand, two horizontal tubes, and a prism arranged to take the light coming from one and to pass it into the other.

Light falls upon the prism through one tube known as the "collinator tube." A slit at the end of this tube admits a narrow ray of light which, by means of a convex lens in the other end of the tube, is made to fall upon the prism with its rays parallel. In passing through the prism the ray of light is dispersed by unequal refraction, giving the spectrum. The spectrum thus produced is examined by the observer through the

[15] Norsk Magazin for Laegevidenskaben, September, 1911, lxxii, No. 9.

other tube, which is a telescope. When the telescope is properly adjusted the rays entering from the prism produce a clear picture of the spectrum. If the light used is lamplight, then the spectrum will be continuous, the colors gradually merging one into the other from red to violet. If sunlight is used the spectrum will be crossed by a number of narrow, dark lines known as "Fraunhofer lines." The position of these lines in the solar spectrum is fixed, and the more distinct ones are designated by the letters of the alphabet, A, B, C, D, E, etc.

If, while using artificial light or the solar spectrum, a solution of any substance which gives absorption bands is placed in front of the slit so that the light is obliged to traverse it, then the spectrum, as observed in the telescope, will show one or more narrow or wide, black bands, which are characteristic of the substance examined and which constitute its absorption spectrum. The position of these bands may be located by describing their relation to the Fraunhofer lines.

While the cost of the spectroscope may prohibit its use by the general practitioner, it is to be found in many laboratories, and its use in certain cases, particularly in poisoning, is absolutely essential, since it enables the student to arrive at definite conclusions which cannot be reached in any other way.

For ordinary investigation the pocket spectroscope is all that is required. The detection of CO-Hb and of methemoglobin, the first occurring in carbonic-acid poisoning, the second in the various forms of intoxication, particularly with chlorate of potash. The smallest amount of hemoglobin or its derivatives may be demonstrated with certainty by this means in cases where ocular examination would leave uncertainty.

The determination of CO-Hb (carbon-monoxid hemoglobin) may present difficulty on account of the similarity of the spectrum to that of O-Hb (oxyhemoglobin). The differentiation is only certain when we observe that the line of CO-Hb does not appear on the addition of reducing agents—for example, ammonium sulphate. The CO-Hb is a strong combination, while, on the other hand, the O-Hb is changed by the reagent to that of reduced hemoglobin.

BACTERIOLOGIC EXAMINATION.

Both diagnosis and prognosis may, in some cases, be helped by a bacteriologic examination of the blood, as there are quite a number of diseases in which the pathologic agents enter the blood. Diagnosis may thus be corroborated by a simple microscopic investigation of the blood, as in relapsing fever, malaria, and syphilis. In other cases, because of the small total number of organisms, or of the presence of others due to contamination, it is advisable to proceed by the more tedious but more certain bacteriologic methods. (Tubercle Bacilli, Method of Rosenberger, etc., see page 360.)

THE CLINICAL VALUE OF BLOOD-CULTURES.[28]

"The blood-cultures are most likely to be of value in the following diseases: Typhoid fever, pneumonia, other forms of pneumococcic infections, and the whole group of septic cases in which the sepsis is associated with wounds, pelvic diseases, abortion, puerperal infection, endocarditis, and local diseases of the throat and many other regions.

"In infections with typhoid bacillus, the pneumococcus, staphylococcus, and streptococcus, the results are usually positive.

"In a case of typhoid the examinations along bacteriologic lines are most likely to be successful between the latter part of the first and the middle of the third week. In sepsis due to the pyogenic cocci positive results are obtained in the majority of cases.

"Blood-cultures may clear up doubt in the presence of typhoid fever or pulmonary tuberculosis. They may also be of considerable use in obscure infections in childhood."

The Specimen.—In the preparation of blood-cultures it is advisable to puncture a vein in the arm under strict aseptic precautions, to withdraw one or two cubic centimeters of blood, and to proceed immediately to prepare cultures or proceed with animal experiments.

The technic involved in this study is difficult and requires an expert knowledge of bacteriologic methods. The author re-

[28] Edsall: Quoting Longcope and Evans, Jour. Amer. Med. Assoc., xlvii, 1906, p. 1136.

fers the reader to more extensive treatises and suggests that this work be left to the experienced worker in bacteriology.

COAGULATION TIME OF THE BLOOD.

Much interest has been manifested recently in the theoretical and clinical value of the study of the coagulation time of the blood. A review of literature shows a large number of methods, together with their modifications, which have been devised in an effort to render this property of the blood of practical clinical value. This review shows that the results obtained by competent observers using the latest methods and apparatus have sufficient similarity to form an accurate clinical basis upon which confidence may be placed. The apparatus devised for determining the clotting time are of three distinct types: 1. The capillary tube which forms the basis of the Wright method. 2. The apparatus in which corpuscular motion is observed to cease under a current of air, as in the methods of Russell and Brodie and of Boggs. 3. Those dependent upon the contour of the drop of blood.

This discussion will be limited to a selected few of the many methods employed, because a multiplicity of methods tends to confuse the worker, and because the methods following have been found by the author to fulfill all clinical requirements.

It is essential in the study of the clotting time of the blood to bear in mind the various factors entering into and modifying the results of these observations. According to Dorrance and others, these may roughly be classed as extrinsic and intrinsic. The extrinsic factors are as follows:—

(*a*) Foreign body, as dust or dirt.
(*b*) Area of surface contact.
(*c*) The air evaporation (humidity).
(*d*) Temperature.
(*e*) Size and shape of drop.
(*f*) Mechanical disturbances.
(*g*) Determination of end point (personal equation).

The intrinsic factors are as follows:—

1. Those affecting the blood.
   (*a*) Viscosity.
   (*b*) Blood-pressure.

(*c*) Leukocytes.
(*d*) Specific gravity and anemia.
(*e*) Chemical composition of blood.

2. Physiologic conditions of the body.
(*a*) Sex and age.
(*b*) Menstruation period.
(*c*) Time of day.
(*d*) Diet.

To this may be added the region from which the blood is drawn, the shape, size, and depth of the puncture, contact with vital tissues, and accidental contact with previously shed blood or an old clot.

THE SPECIMEN.

This should be taken midway between meals, and not after the ingestion of drugs, which may influence coagulation time. The tips of the finger or the lobe of the ear should be thoroughly cleansed and dried, and a sufficiently large puncture made with a flat-bladed instrument (Daland lancet) to insure free and sufficient flow.

METHODS OF DETERMINATION.

Milan's Method.—Place a drop of blood upon a large glass slide or in the center of a watch-crystal. After a minute or two gently tilt the glass from side to side. This is continued at short intervals until the drop of blood, which at first assumed a pear shape when the glass was tilted, is seen to assume the form of a blunt and symmetrical cone. This change in the character of the drop indicates the completion of coagulation. The average time of coagulation of normal blood by this method is about five minutes.

This method is at best imperfect and uncertain, since none of the modifying factors are controlled.

Method of Russell and Brodie.—This method requires a microscope. The coagulometer consists of a small, moist chamber with a glass bottom, which can be placed upon the stage of the microscope. This is fitted with a truncated cone of glass, which projects downward into the moist chamber. The end surface of this cone is of definite size (about 5 millimeters

in diameter), and on it is placed the drop of blood, care being taken to see that the drop of blood only just covers the surface; hence is always of the same size. The cone is then quickly fitted into the moist chamber to prevent alterations due to drying, temperature, etc. Through the side of the moist chamber projects a fine tube, through which, by means of a hand-bulb, a gentle stream of air can be projected against the blood. The preparation of the slide being completed, the drop of blood is observed with the low power of the microscope.

The blowing should be done at as long intervals as possible, and also very gently. The corpuscles will first be seen to move freely as individuals; later, as coagulation begins, the corpuscles will no longer move freely in the drop, but the drop will begin to change shape *en masse*. Finally, there will occur only elastic

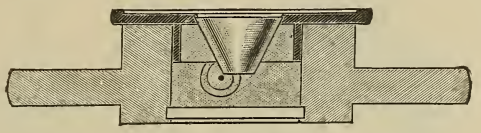


FIG. 19.—BOGG'S COAGULOMETER. (A. H. T. Co.)

motion, and the part of the drop displaced by the current of air will spring back to its original position as soon as the current of air ceases. This is to be taken as the terminal point, as now only can the clot be demonstrated by quickly removing the cone and touching the drop to a piece of dry filter paper.

Recently this instrument has been improved upon by Boggs, who has substituted a metal tube and an improved cone (see Fig. 19).

With the improved instrument Emerson ("Clinical Diagnosis") reports normal variations in the coagulations between three and eight minutes, with an average of five minutes eight seconds.

Method of Dorrance.[17]—The coagulometer of Dorrance is composed of an 8-ounce thermos bottle without the silver lining, an aluminum stand, and two rubber corks which fit snugly into the bottle. One cork has a hole in it for a thermometer which

[17] The description of this instrument is taken from the deviser's article appearing in the American Journal of Medical Science, October, 1913, No. 4, p. 562.

registers up to 140° F. The other cork has four openings, each of which is lined with a brass flange. Into each of these openings a glass rod fits. These rods are 4½ inches long, with a diameter of 8 millimeters. One end is cone-shaped, with a flat

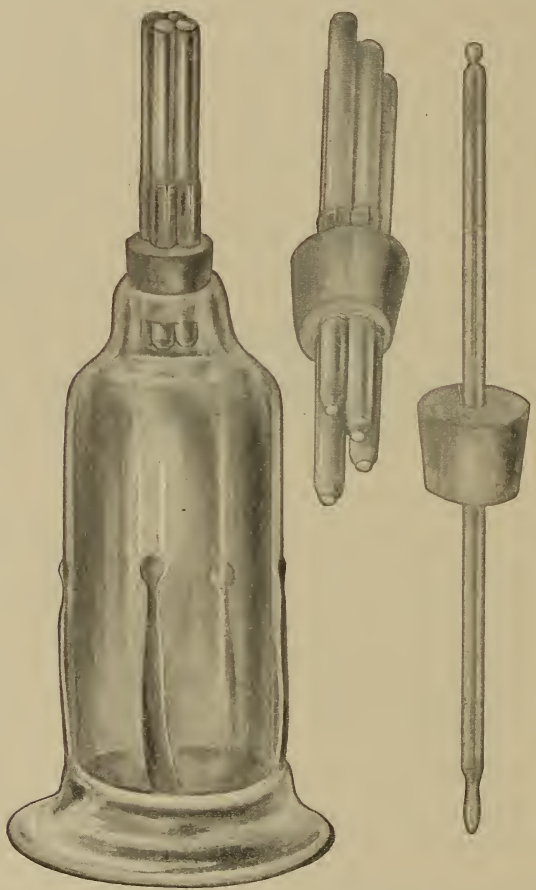


FIG. 20.—DORRANCE'S COAGULOMETER.

tip 4 millimeters in diameter. The other end is slightly bulbous, to prevent the rod from slipping through the flange.

METHOD OF APPLICATION.—The thermos bottle is filled with water at 98° F. to within one inch of the top. It is then covered

with the cork containing the thermometer to note any change in the temperature of the water. The rods within the second cork are first scrubbed with soap and water and then cleansed with alcohol and finally with ether. This cork is then substituted for the one containing the thermometer, so that the rods will become heated to the temperature of the water. This is done while the finger of the patient is being prepared. The rods during the time that they are in the bottle heating up to the temperature of the water will have collected considerable moisture, and it is therefore necessary to wipe them dry before collecting the blood, else the moisture will dilute the drop of blood. The finger is given a good stick, so that it bleeds without pressure. The first drop of blood is wiped off. As soon as another drop begins to appear the cork containing the glass rods is removed from the bottle and the rods are wiped dry. Then with care each one of the rods is placed in contact with the drop of blood. If done carefully the same sized drop will be taken up by each rod. From the time of the appearance of the drop until the blood is collected and the cork replaced in the bottle should not be more than ten seconds.

The end point with this instrument is determined in three ways: 1. In explaining the end results we shall number the glass rods in the order in which the blood was collected upon them. At the end of two and one-half minutes rod No. 1 should be pushed down into the water. It will be noted that all the blood falls off the end of the glass rod and breaks up into a fine cloud. Rod No. 2 is introduced into the water at the end of three and one-half minutes. There will still be considerable falling off of the blood, but when it breaks up the particles will be coarser and there will remain a small amount on the end of the rod. Rod No. 3 is introduced into the water at the end of four minutes. There will still be noted some dropping off of the blood, and there will be found a larger amount of blood adherent to the end of the rod. Rod No. 4 is introduced into the water at the end of four and one-half or five minutes, depending upon the action of the former rods. There will be a slight falling off, but the greater part will be found to be adherent to the end of the rod if coagulation has taken place. The fact that we start at two and one-half minutes from the time of the appear-

ance of the drop is only an arbitrary factor. The test can begin at any time and the rods be introduced at any time that the operator desires.

2. The cork containing the four rods is now removed from the bottle and held up to the light, with the end on which the blood is adherent nearest to the eye. On looking through the rods the following results will be found: Rod No. 1 will be clear. On Rod No. 2 will be observed a slight reddish tinge. On Rod No. 3 this reddish tinge will be somewhat deeper in color and the end of Rod No. 4 will be almost covered with a red clot.

3. The end of each rod is blotted with filter paper or blotting paper, as shown in Fig. 2. The blot made by Rod No. 1 will be practically clear. Rod No. 2 will show a slight red color, Rod No. 3 will show a more distinct red, and Rod No. 4 will show a clot. If the last rod does not show the blood to have coagulated, the test should be repeated, with the first rod introduced at four minutes and the others at either one-half minute or one-minute intervals.

It is a well-established fact that the first drop does not coagulate as rapidly as the later ones, and, as we want to know the shortest time, we take about the fourth or fifth drop. Blood that is taken from the veins and which does not come in contact with the tissue juices does not give the true clinical coagulation time. The tissue juices are one of the most important factors in the causation of coagulation.

Pathology.—The pathologic significance of coagulation time clinically is as yet indeterminate. In typhoid fever it is variable, being more rapid especially in cases with thrombosis. It is rapid in leukemia, diabetes, endocarditis. It is delayed in hemophilia, purpura hæmorrhagica, lymphatic leukemia, and splenic anemia. In diseases of the liver and gall-bladder the decreased coagulation seems to vary directly with the depth and intensity of the jaundice. Of the acute infections, lobar pneumonia and septicemia show the most consistent and longest delay of clotting.

On the other hand, T. Addis,[18] found in examining a large and varied group of diseases that in 70 per cent. the coagulation time was not disturbed. Moderate loss of blood has no effect

[18] Edinburgh Med. Jour., July, 1910.

on coagulation, but after large hemorrhage the coagulation time is accelerated.

Rudolph[19] says that of the effects of drugs upon the coagulation he found calcium lactate did not appear to have any effect, and that citric acid appeared to have a slight retarding influence.

In the study of coagulation it must be remembered that the personal equation and large variety of apparatus affect the determination of the end point, and that it is difficult to make an exact comparison of the results of different workers. It would seem advisable that some uniform procedure should be devised and adopted, thereby minimizing the effect of the factors of error, and yet which would be sufficiently practical for all clinical purposes.

The work of Robertson and Illman, while having been done some years ago, still remains unchanged. They employed the cumbersome Wright apparatus and obtained the following results, which are based upon many observations of a large number of patients:—

Specific meningitis	3.10	minutes.
Coal-gas poisoning	4.05	"
Gastric ulcer	4.10	"
Salpingitis	5.15	"
Alcoholic gastritis	3.10	"
Apoplexy	3.05	"
Carcinoma	4.35	"
Jaundice	6.30 to 8.30	"
Rheumatism	3.00 to 4.00	"
Pneumonia	2.47	"
Nephritis	2.25	"
Diabetes	2.55	"
Exophthalmic goiter	2.45	"

VISCOSITY.

The great activity in the study of blood-pressure during the past decade has served to emphasize the importance of blood composition in relation to its passage through the vessels. In the section on blood-pressure will be found discussed the factors determining both normal and pathologic blood-pressure, and

[19] New York Medical Journal, August 13, 1910.

among them we find two which are chiefly concerned in determining the peripheral resistance to the circulation of the blood. These are: 1. The ever-changing caliber of the arterioles and capillaries. 2. The viscosity of the blood.

According to Huertel, the work done by the heart of a dog is four times greater than it would be if the vessel were filled with water. This difference is due to the viscosity of the blood, which is a physical property depending on the freedom of motion between the particles which constitute the blood and which, according to different investigators, in the normal human is 3.5 to 5.5 times less than that of water. We are not particularly concerned with the importance of the individual factors which combine to produce the normal viscosity of the blood. Some of this data is still in the speculative stage, and has not yet been sufficiently studied to be of clinical importance.

A rather interesting theory is that of Adams, who maintains that the viscosity is dependent primarily upon the amount of gas which the blood contains, saturation with carbon dioxide producing a maximum of viscosity. If the carbon dioxide be replaced by oxygen then the internal friction is reduced to a minimum.

There is probably a relation between the viscosity and the fibrin in the blood, as it has been noted that viscosity and coagulability increase and diminish *pari passu* within certain limits. We do not believe that the viscosity of the blood is altogether dependent upon the specific gravity because of the fact that high viscosity may coexist in fevers with a low specific gravity. For definite physical reasons, the amount of protein and salt contained in the plasma greatly influences the viscosity, and it is probable that among drugs the iodides are the most important, as they appear to have a powerful effect in diminishing viscosity.

Factors Affecting Viscosity.—The viscosity of a normal individual varies according to age and sex. But there exists a difference of opinion among investigators as to the average viscosity for different ages of healthy individuals. In infants it probably varies between 3.2 and 3.6, reaching 3.9 to 4.2 at about 10 years, being between 4.2 and 4.5 between 12 and 20. The average of all investigators places it between 4.5 and 5.1.

Blood viscosity is a relatively constant factor, showing that variations above 4.4 and 5.3 in men and 3.9 to 4.9 in women must be considered pathologic (Sachs). It is believed that viscosity is slightly lowered in males after the fiftieth year. Blunschy states that muscular exertion, when severe and prolonged, reduces viscosity, but that when mild and comparatively brief, even when causing perspiration, it increases it.

It is generally believed that meat-eaters have a relatively higher viscosity than vegetarians, although the difference is but slight. The ingestion of large quantities of water and food appears to temporarily influence viscosity.

Principle of Determining Viscosity.—The fundamental principle in any apparatus for determining the viscosity of the blood is that of forcing blood through a given length of capillary tubing under a definite pressure and temperature and comparing the time consumed with that required to force distilled water through the same tube under like conditions. For this purpose McCaskey has devised a very simple and practical apparatus, which, while devoid of absolute scientific accuracy, fulfills the requirements of clinical medicine.

Method of McCaskey.—The instrument consists of a capillary pipette made from a piece of glass tubing with a lumen of 5 millimeters. One end is tapered so that the rubber bulb can be easily fitted on it. The other end is drawn out into a fine capillary tube, the exact size of which must be determined by experience. This capillary tube is substantially the same as the capillary pipette used in opsonic work. A convenient length for the capillary portion of the tube is about 6 inches, and it should be made so that distilled water will pass through it under the negative pressure of the bulb in about five or six seconds. A fine inkmark is made at 5 inches from the tip just before the lumen of the capillary tube begins to enlarge, and each experiment terminates at the moment when the moving column of blood reaches this mark. It is first necessary to "standardize" the tube with distilled water, which constitutes the unit of comparison. The rubber bulb is taken between the thumb and index finger and compressed until its inner surfaces are in contact. It is important that this technic should be accurately followed. If this be done there will not be any sub-

stantial variations in the negative pressure of the same bulb in different experiments. If, however, the bulb is compressed flatly, say, between the palmar surfaces of the thumb and two fingers, the negative pressure will be increased from 40 to 50 per cent. and the results entirely vitiated. The negative pressure will vary with different bulbs. The bulb thus compressed is fitted over the capillary pipette at the end of the tube and the pressure released. Air flows into the capillary tube under a constant negative pressure. Holding a stop-watch in the other hand, the end of the pipette is immersed in distilled water contained in a watch-crystal and the stop-watch started at the same instant. The watch is stopped when the column of water reaches the mark. This is repeated three or four times, and, if there be any variation in the time, the mean of the several observations is taken and a label is attached to the tube showing the result and also the temperature of the room. The latter is important because the viscosity falls about 2 per cent. with each degree centigrade of rise of temperature; and if the observation on the blood is made at a temperature substantially different from that in which it was standardized with distilled water, corresponding corrections must be made.

The *clinical observation* of the blood viscosity is made as follows: One arm of the V-shaped tube (made of glass tubing with a lumen of 1.5 millimeters) is partially filled with blood by holding it against the under surface of a drop of blood exuding from the side of the tip of the finger or from the lobe of an ear. Five or 6 drops are required. This tube is then held by an assistant, or in a suitable clamp attached to the arm in which the blood has been collected, leaving the clear arm of the tube free for observation. The bulb and capillary tube are arranged precisely as heretofore described for the experiment with distilled water, and the end of the capillary tube is passed down the clean arm of the V-shaped tube until it comes close to the surface of the specimen of blood. Then, while holding the stop-watch in the other hand, it is quickly plunged into the blood and the stop-watch is started at the same instant, the watch being stopped as before when the moving column of blood reaches the inkmark. It is then simply a matter of dividing the time required by the blood to pass through the capillary tube by the

time required by the distilled water to determine the viscosity. If, for example, with a particular pipette, it would necessitate twenty-five seconds for the blood to reach the mark, while distilled water requires five, then the viscosity would be five. This observation can be made in three minutes and is sufficiently accurate to serve the purpose of clinical medicine. Sometimes the blood coagulates so rapidly that only a single observation can be made. This can be obviated by placing a crystal of hirudin into the V-shaped tube, as recommended by Bence and Determann. The blood will then remain fluid for twenty or thirty minutes. The greatest objection to the use of hirudin is that it is very hygroscopic and must be kept hermetically sealed, or in a chamber with a desiccator. Its use, however, does not change the viscosity in the least or even modify rouleau formation, so that it is available for other purposes in which slow coagulation is desirable as well as the determination of viscosity. The viscosity tube can be cleaned in the usual way with water, alcohol, and ether. If the tube is spoiled it can be easily and cheaply replaced.

Method of Denning and Watson.—A second method, which is very simple, the apparatus for which may be obtained on the market, is as follows:[20] It consists of a J-shaped capillary tube, the long arm of which measures 6 and the short arm 2 centimeters in length. The upper end of the former is molded so as to form a funnel-shaped receiver, and in the course of the short arm the tube is blown to form an elliptical bulb. The points where the capillary bore meets and leaves the bulb are marked on the glass. To use the instrument the lobe of the ear is pricked (aseptic precautions being observed), and the instrument, which has been warmed to the body temperature, is held under the exuding drop of blood, so that it flows into the receiver. The flow down the tube is carefully watched until it reaches the mark indicating its entrance into the bulb. The time is immediately noted on a stop-watch, and again when the bulb is filled, when it is read off to the fraction of a second. The instrument is calibrated for water, the viscosity of which is known, and the time value is marked on the back of the tube.

[20] A. Denning and J. H. Watson: Lancet, July 14, 1906; and Liverpool Med. Chir. Jour., July, 1906, p. 46.

Factors Affecting Viscosity.—Carbon dioxide increases viscosity. For this reason asphyxia more promptly affects viscosity than any other known factor, whether this be general, or a local condition due to blood stasis.

Experiments in asphyxia have shown viscosity to increase from 4.63 to 8.83 (Determann). A viscosity factor of 12 was obtained by Trumpp in a newborn infant in blue asphyxia. Slight exertion in persons with cardiovascular disease markedly increases viscosity. A number of observers state that an increase in viscosity is an early sign of cardiac insufficiency and suggest the importance of this observation as an aid in early diagnosis. In this connection it has been stated that patients with increased viscosity will, as a rule, die, while those with a low viscosity can be given a better prognosis.

Venesection lowers viscosity, and its value as a therapeutic measure is probably in a large measure dependent upon this relief afforded the heart. Intravenous administration of salt solution reduces viscosity; so also does hemorrhage. The effect of the former is of short duration, while that of the latter quite lasting. Viscosity is high in pneumonia and in emphysema and low in anemia and impoverished blood conditions.

The relation of viscosity to renal diseases is particularly important. It is now believed that in chronic interstitial nephritis the viscosity is low, even in the presence of a high blood-pressure. From the clinical standpoint a rather important observation is that of Trumpp, who observed a rather high viscosity in children with alimentary intoxication. From the surgical standpoint, the viscosity is high in clean abdominal operations during the first two or three days, and gradually falls to and below the normal, returning to normal at the end of a week. The same change is apparently present in septic cases, where the inflammation is relieved, but remains high if the infection persists. In typhoid fever the viscosity is usually low, while in meningitis it is generally increased. The effect of drugs is most marked. Alcohol greatly increases the viscosity. Caffeine also increases the viscosity, while camphor reduces this factor. Chloroform and ether do not seem to have any effect on viscosity.

IV.

SPHYGMOMANOMETRY AND SPHYGMOGRAPHY.

A. SPHYGMOMANOMETRY.

Capillary Blood-pressure.—The pressure of the blood in the capillaries is low, because of the resistance offered to the progress of the blood by the fine bore of the vessels, and because of the relatively large cross-sectional area of all the capillaries compared to that of the aorta and great vessels.

The capillary pressure has been found to be much lower than in the arteries, and considerably higher than the pressure in the great veins. This pressure has been found to equal that required to sustain a column of from 24 to 54 millimeters of mercury.[1] Clinically the capillary blood-pressure is not of sufficient importance to warrant a further discussion here.

Terms and Definitions.—The Pulse: The pulse is the rhythmically recurring impulse propagated by the systole of the left ventricle and palpable throughout the arterial system.

Arterial Pressure.—By arterial pressure is meant the degree of force exerted by the blood within the vessel. It is primarily dependent on the strength of the heart as measured by its rate and by the volume of blood expelled at each systole, balanced by the elasticity of the vessel walls and capillary resistance.

The Systolic Pressure (Fig. 21).—The systolic pressure, as indicated by the sphygmomanometer, represents the pressure within the vessels at the time of systole of the ventricles.

The Diastolic Pressure (Fig. 21).—The diastolic pressure represents the ebb to which the arterial pressure falls during cardiac diastole.

The Pulse-pressure, Range or Amplitude (Fig. 21).—The arterial pulse is caused by variations in pressure within

[1] Amer. Textbook of Physiology, p. 377.

the arterial system caused by the intermittent pumping action of the heart. The difference between systolic and diastolic pressures, *i.e.*, the variation in pressure occurring within the vessel during a complete cardiac cycle, is termed the pulse-pressure. This figure is obtained by subtracting the diastolic from the systolic pressure. The normal pulse-pressure ranges between 20 and 40 millimeters of mercury.

Variations in the pulse-pressure in the same individual constitute a most important part of the study of blood-pressure.

It is theoretically possible that the pulse-pressure should be influenced in at least three ways: 1. An increase in the amount of blood delivered at each beat of the heart would tend to increase the difference between systolic and diastolic pressures. 2. A

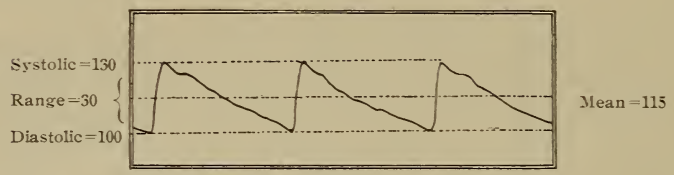


FIG. 21.—NORMAL PULSE TRACING: SHOWING RELATION OF SYSTOLIC, DIASTOLIC, PULSE-PRESSURE AND MEAN. PULSE-PRESSURE EQUALS 30.

rapid emptying of the vessels, the cardiac output remaining the same, would tend to increase this difference. This would occur independently of whether the blood was passed onward into the capillaries or was regurgitated into the ventricle. 3. Rigid vessel-walls would increase pulse-pressure. If the arteries were rigid tubes, the heart at each systole would be compelled to move the blood in the arterial system as a whole, while during diastole the flow would cease. There would thus be an increase of pressure during systole, while during diastole it must fall rapidly toward zero.

THE MEAN PRESSURE.—The mean blood-pressure is valuable chiefly as an indication of the amount of strain to which the heart and larger vessels are subjected. It varies with the pulse-pressure, the systolic pressure, and the diastolic pressure.

To obtain the mean pressure, divide the sum of the systolic and diastolic pressures by two, or add half of the pulse-pressure to the diastolic pressure (Fig. 21).

Pathologically, the pulse-pressure increases in organic diseases of the kidneys, in arteriosclerosis, and in aortic insufficiency. It diminishes from other organic diseases of the heart, affecting the valves or myocardium.[2]

Significance of Pulse-pressure.—The normal pulse-pressure indicates a normally acting heart, a proper systolic output, and a normal vascular distribution of blood. Any condition which interferes with the normal flow of blood from the capillaries will tend to increase the systolic pressure, and will produce a greater pulse-pressure. We find therefore an increased pulse-pressure in arteriosclerosis. The increased systolic output of the large heart in aortic regurgitation produces, during compensation, a very large pulse-pressure. According to Gerhardt, the systolic blood-pressure in broken compensation may be high, but the pulse-pressure is always diminished and becomes greater, according to the relationship of compensation. An increased pulse-pressure in the absence of any other explanation is very suggestive of organic disease of the kidneys. Myocardial degeneration of all kinds is productive of a reduction of the pulse-pressure, and the mean of this is a fair estimation of the state of the myocardium, upon which is based the work test of Graüpner (test on page 119).

The Principle of the Sphygmomanometer.—Vital tissue is perfectly elastic. Therefore any pressure applied to the surface of the body will be directly transmitted to the underlying structures without loss of force. It is upon this principle that the indirect method of measuring the blood-pressure is based.

Pressure is applied to an accessible part of the body over a large blood-vessel, such as the brachial. If the amount of this pressure is sufficient to overcome the pressure of the blood within the vessel, the vessel will be collapsed and the pulse prevented from passing beyond it. If the amount of the compressing force is measured and expressed in definite terms of weight (as millimeters of a column of mercury) then we can, by applying just sufficient pressure to collapse the vessel, measure the amount of force exerted by the blood in preventing this collapse.

In practice the compressing force is obtained by a cautery-bulb or a small hand-pump, and applied to the arm by means of

[2] Eichberg: Jour. Amer. Med. Assoc., Sept. 19, 1908.

a hollow, flat rubber bag. This is applied about the arm and held there by some form of inelastic cuff. A tube communicating with a mercury or an aneroid manometer measures the amount of pressure applied over the vessel.

The Sphygmomanometer.—The apparatus devised for measuring blood-pressure may be divided into three classes: 1. The spring. 2. The mercury. 3. The aneroid.

1. The spring type of instrument represents efforts to pro-

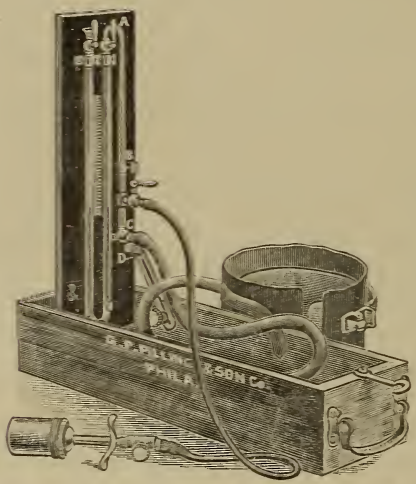


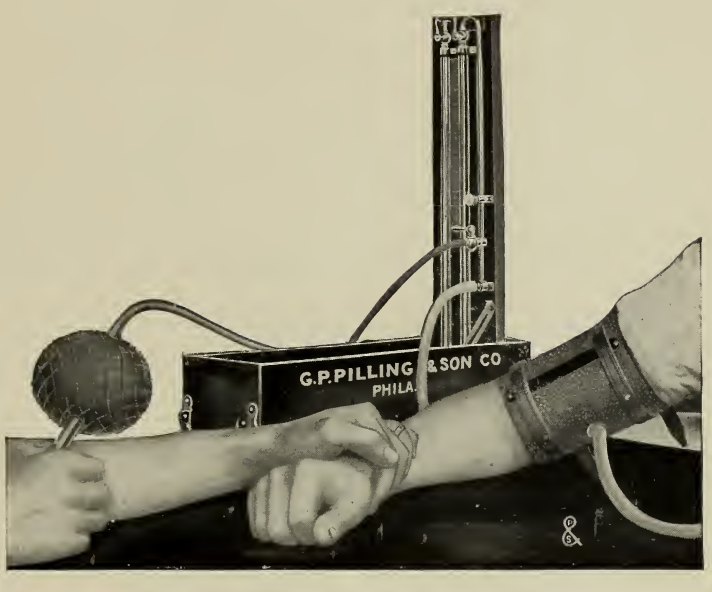
FIG. 22.—FAUGHT'S MERCURY SPHYGMOMANOMETER.

duce a clinical instrument of small size and minimum cost; so far these have not measured up to the requirements of clinical medicine, and, therefore, cannot be recommended for clinical use, as they are neither dependable nor accurate.

2. The mercury type of apparatus is divided into two classes: One of these employs a vertical tube into which the mercury column is forced from a large containing chamber in the base of the instrument. The pressure is measured in millimeters of mercury on an appropriate scale attached to the vertical glass tube.

The other employs a glass tube (similar to that first used by Poiseuille) bent in the form of a "U" with the open ends up. This tube is partly filled with mercury and one end connected

PLATE III



Sphygmomanometer in Position for Observation.

by means of suitable tubing with the compression part of the apparatus. The pressure is measured upon a suitable scale placed between the two limbs of the tube, and is represented by the difference in the height of the mercury in the two limbs of the "U" tube.

The mercury sphygmomanometer (Fig. 22) bearing the author's name is modeled after the type of apparatus employing the "U" tube and is designed to overcome the many shortcomings of the earlier instruments and to furnish an instrument which is

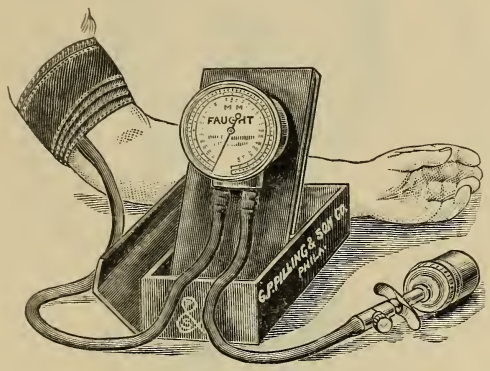


FIG. 23.—FAUGHT'S CLINICAL SPHYGMOMANOMETER.

easy to use, difficult to get out of order, accurate and as light and portable as is compatible with exactness and strength.

The mahogany case, which encloses the complete apparatus, including the arm-band and pump, measures 4 x 4½ x 16 inches and weighs 3 pounds and 9 ounces. The lid is hinged at one end and when raised supports the working parts of the apparatus. A spring check allows the lid to be raised to a vertical position, where it is automatically held locked during observation.

The "U" tube is provided with a scale, which has been arranged to give the reading directly in millimeters of mercury, each space representing 2 millimeters Hg. The range is from 0 to 300.

A special and distinctive feature of the apparatus is the means of preventing loss of mercury from the manometer tube when the instrument is not in use. This is accomplished by

means of two small cocks placed at either extremity of the "U" tube, and which are kept closed when the apparatus is not in use.

By eliminating all detachable parts, the time required to make the reading is reduced to a minimum, the only preliminaries to the test being to lift the lid, open three cocks and attach two tubes to their respective nipples.

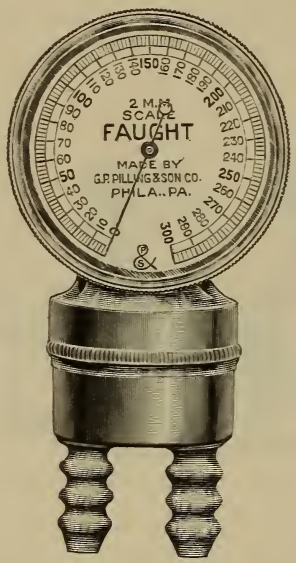


FIG. 24.—ACTUAL SIZE POCKET INDICATOR.

3. The aneroid sphygmomanometer represents the acme of pressure-measuring instruments yet made. This instrument is at once small, compact, reliable, and accurate, and, when properly used, almost indestructible.

The principle is that of the aneroid barometer except that in the sphygmomanometer of this type the fixed pressure within the chambers (see Fig. 25) is the atmospheric pressure (instead of a vacuum, as in the barometer), while the variable pressure is that produced within the apparatus by means of the pump and which can be changed at the will of the operator. As characteristic of this type of instrument, the Faught aneroid may be described as follows:—

The Faught Pocket Sphygmomanometer.—This instrument consists of a gold-plated aneroid gauge with a white-enameled dial, each space of which represents 2 millimeters and will give readings from zero up to 300 millimeters. This together with the flexible arm-band and metal pump constitute a

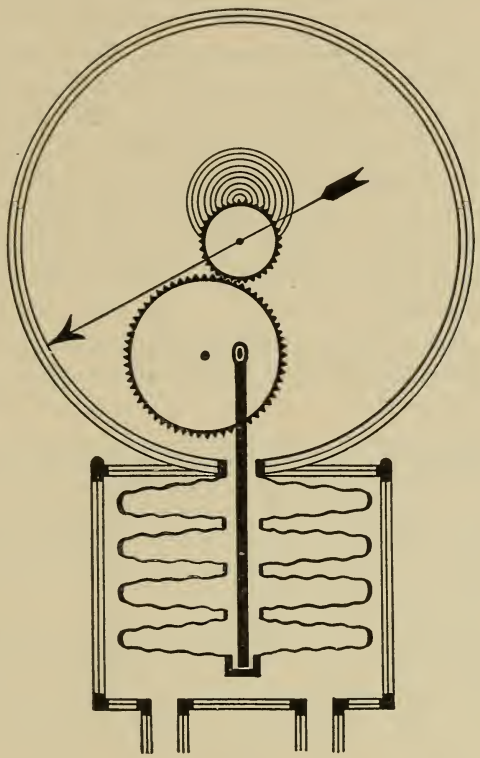


FIG. 25.—ENLARGED DIAGRAM SHOWING THE PRINCIPLE AND THE WORKING PARTS OF THE AUTHOR'S POCKET INDICATOR.

very simple and practical sphygmomanometer, which, with its carrying case, can fit very easily in the coat-pocket. This instrument fulfills all the demands of the blood-pressure test, giving accurately both the systolic and diastolic readings, from which can be computed the pulse-pressure and the mean. It has been in practical operation sufficiently long to prove not only that it is accurate, but that it maintains its accuracy,

making it unnecessary to repeatedly compare the scale with the standard mercury column. The rubber portion of the arm-band measures 5 x 9 inches, and so conforms with the requirements of the best authorities. The apparatus can be applied and removed in a very short time, and complete observations can be made in less than two minutes. It is not affected by temperature or atmosphere, since when the apparatus is at rest the pressure on both sides of the diaphragm is equal. When using this apparatus, the method is the same as directions under the mercury sphygmomanometer, excepting that the needle valve is placed upon the pump instead of on the indicator.

This apparatus is constructed with two dials: the regular pocket apparatus (see Fig. 24) and the clinical apparatus (Fig. 23), which possesses a 3½-inch dial and a scale reading to 350 millimeters, useful for hospital and bedside work, especially in ward-class teaching.

The principle involved in sphygmomanometry being the same, regardless of the type of apparatus employed, a single description will suffice.

To Operate the Sphygmomanometer.—The patient should be in a comfortable, easy, and relaxed position; repeated and subsequent observations of the same patient should be made, for the purposes of comparison, under as nearly the same conditions as possible. This refers especially to arm used, and to the position of the patient (see below).

The arm-band is wrapped snugly around the bared arm of the patient above the elbow. The tube emerging from the cuff or arm-band is attached to the indicator by one nipple and the pump connected by a short piece of stout tubing to the other nipple. The escape valve, whether it be on the indicator or attached to the pump, is closed.

This arrangement forms a continuous closed pneumatic system communicating freely with the manometer tube of the instrument. Now, when pressure is raised in the arm-band, by the pump, the amount of force exerted is shown on the dial by the pointer, or on the scale by the level of the mercury; the readings in either case, being in millimeters of Hg, are therefore comparable.

TO OBTAIN THE SYSTOLIC READING.

Method by Palpation.—With one hand find the pulse at the wrist of the arm to which the arm-band has been applied. The fingers should be in a comfortable position and should under no circumstances be moved during the observation. Care should also be observed that the pulse is not cut off by undue pressure of the palpating fingers.

While the pulse is thus under observation, the pressure in the apparatus is raised by means of the pump until the pressure within the constricting band is sufficient to prevent the pulse from reaching the wrist. Now by a fraction of a turn in the escape valve the pressure in the system is slowly released. During this part of the procedure, a close watch should be kept upon the position of the needle or the height of the mercury column and for the return of the first pulse-beat at the wrist. The systolic reading is made at the instant the first pulse-beat is felt to pass the constricting band and reach the wrist. It is advisable to repeat this procedure a few times to check the correctness of the finding.

AUSCULTATORY METHOD OF OBTAINING SYSTOLIC PRESSURE.

As the auscultatory method of blood-pressure reading is now the method of choice, the reader is referred to the following pages, in which this technic is described in detail. Besides being more accurate, the auscultatory method gives definite results in every case. It must be remembered, however, that when this method is employed the systolic readings will average a few millimeters higher than when made by palpation.

TO OBTAIN THE DIASTOLIC PRESSURE.

There are four recognized methods of reading the diastolic pressure:—

1. The visible.
2. The palpatory.
3. By means of special indicators.
4. By auscultation.

Experience and experimental study have conclusively demonstrated the first three to be unreliable. The figures obtained by

them do not always indicate the correct pressure, except possibly the complicated and expensive instruments of Uskoff and Erlanger. For this reason the discussion here will be confined to a consideration of the auscultatory method, first advocated about eleven years ago by Korotokow.

The Auscultatory Method of Blood-pressure Reading.— Korotokow discovered that, by placing the bell of a small stethoscope over the lower end of the brachial or the beginning of the radial artery, a series of pulse-tones could be produced by the pressure of the sphygmomanometer-band in position on the arm above it, and that these sounds were not of cardiac origin, but were the direct results of the effect of compression of the artery by the cuff. He described, in all, three phases or sounds, and discovered that the first occurrence of sound over the artery was simultaneous with the first passage of blood under the cuff, and was, therefore, an accurate indication of the point of systolic pressure. The second phase was a fair indication of heart strength, while the third phase was the sudden disappearance of all sounds and indicated the moment of diastolic pressure.

Subsequent observers, notably Sterzing, Goodman and Howell, and Warfield, have described a further analysis of the pulse tones, and divide them into five.

The tone phases are usually distinct and clear-cut, and bear a definite relation to the difference between systolic and diastolic pressure. For example:—

With a normal systolic pressure of 130 millimeters and a diastolic of 85 millimeters the phases and their average duration are as follows:—

1st phase. A loud, clear, snapping tone which immediately follows a silent stethoscope, and which is the index of systolic pressure. Duration, 14 millimeters.

2d phase. A succession of murmurs, less distinct than the preceding, but yet well distinguishable. Duration, 20 millimeters. This phase is dependent upon cardiac efficiency.

3d phase. This tone resembles the first phase, but is less sharp and distinct. Duration, 5 millimeters.

4th phase. A dull tone, lasting for about 6 millimeters.

5th phase. The disappearance of all sound.

Authorities are not yet as one on the question of the dias-

tolic point, older observers holding the disappearance of all sound to be the correct diastolic point, while later investigators, notably Warfield,[3] hold that the beginning of the fourth phase is the true diastolic point. However, as the difference in reading by these two methods rarely equals more than 6 millimeters, the variation caused by difference of end point is but slight. Time alone will settle this question.

In employing the auscultatory method, much time is saved, and the physician is assured of greater reliability and accuracy in his results, when the one operation serves to give both systolic and diastolic reading; at the same time conveying to the ear much valuable data concerning the condition of the heart and blood-vessels. One point, however, must be borne in mind: many readings in the textbooks and medical literature are based on the first and second methods. The auscultatory method will give readings of a slightly higher systolic pressure and a diastolic pressure of 5 to 10 millimeters lower.

APPLICATION OF THE AUSCULTATORY METHOD.

After arranging the apparatus in the usual manner and raising the pressure to obliteration of the pulse, a stethoscope is placed over the brachial artery below the cuff. As the pressure is gradually allowed to fall, a pulse tone is heard as the circulation commences. This represents systolic pressure. This tone now undergoes a number of changes (described above) until it suddenly becomes very faint and almost immediately disappears. The reading of the sphygmomanometer at this moment represents the diastolic pressure.

Cautions.—To obtain accurate and reliable clinical data with the sphygmomanometer, it is important that some systematic technic be adhered to, and that all observations not only on the same patient, but in all cases, be made under as nearly the same conditions as possible. Attention to detail will eliminate largely the errors arising from such factors as position of the patient, presence of fatigue or mental excitement, arm used for observation, etc. It is also valuable to note the apparatus used, the time of day, the pulse rate, the sex and age of the patient.

[3] Jour. Amer. Med. Assoc., 1913, lxi, 1254.

Care should also be taken to see that the observation is not too prolonged, for the interruption of the circulation in the extremity will, if continued, itself cause changes in pressure.

No Single Reading Should be Accepted When it is Possible to Make More than One.—It is better to see the patient a number of times under varying conditions before deciding what his average blood-pressure is.

THE PULSE-PRESSURE AND THE MEAN PRESSURE.

Having determined the systolic pressure and the diastolic pressure, the diastolic pressure is subtracted from the systolic pressure and the remainder is the pulse-pressure (see Fig. 21, page 102).

To obtain the *mean pressure,* add one-half of the pulse-pressure to the diastolic pressure.

In order to enable physicians to take the blood-pressure readings more accurately and to make them of greater clinical value to the profession as a diagnostic and therapeutic guide, several devices for automatically holding the stethoscope in place on the arm during the observation have been devised. Those having the stethoscope fixed and incorporated in the arm-band are to be condemned, because of the adventitious sounds bound to be produced by slipping of the compression cuff and its retainer, which, heard by the physician, are very confusing, and also because the conception of the idea is faulty, as either the stethoscope must be on the outside of the arm, if the bag is over the artery, or else the bag must be interposed between the stethoscope and the artery, if they are both on the inner aspect of the arm; either arrangement preventing satisfactory work.

The sphygmometroscope of Prendergast,[4] employing a button-like projection of the receiving disc, and a separate fabric band to retain the apparatus, has much to recommend it and in the author's hands has given great satisfaction. Even better than this is the bracelet stethoscope of Dr. H. Stamp, shown at the meeting of the Amer. Med. Assoc. in 1914. This provides an elastic metallic clamp for retaining purposes and by this little device the stethoscope can be applied and removed instantly.

[4] N. Y. Med. Jour., Jan. 11, 1913.

The button on the receiving end snugly fits the elbow bend, and the "all metal" construction does away with all adventitious sounds. This instrument is shown in Fig. 26.

Methods of Recording Observation.—For convenience in study, comparison, and for future reference, it is advisable to formulate and adhere to some method of recording the blood-pressure observations. Charts which have been found satisfactory for this purpose will be found on pages 437 and 438.

The Normal Blood-pressure.—Experimental study and clinical observation have established, within fairly well defined

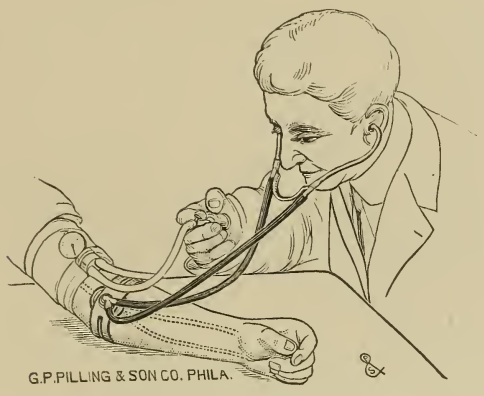


FIG. 26.—STAMP BRACELET IN USE.

limits, the normal blood-pressure in man, and also the extent of what may be termed the *physiologic variation*. That is, the extent to which the normal reading may be modified by age, sex, exercise, time of day, altitude, posture, etc.

Factors which may Normally Influence Blood-pressure Readings.—AGE: There is little difference of opinion among observers that 90 millimeters Hg is the minimum normal systolic blood-pressure in the young adult. The upper boundary is more difficult to establish, because it may be almost impossible to eliminate the possibility of pathologic influences the presence of which are undiscoverable. Excluding all modifying influences, both physical and mental, we may consider that a blood-pressure reading in a young healthy adult which remains constantly above 140 millimeters is abnormal and calls for explana-

tion. Females usually have a slightly lower blood-pressure, as to the amount of which authorities differ; personally I believe that this should not exceed 10 millimeters.

The work of H. P. Woley[5] of examining 1000 healthy subjects between the ages of 15 and 60 years is a very important

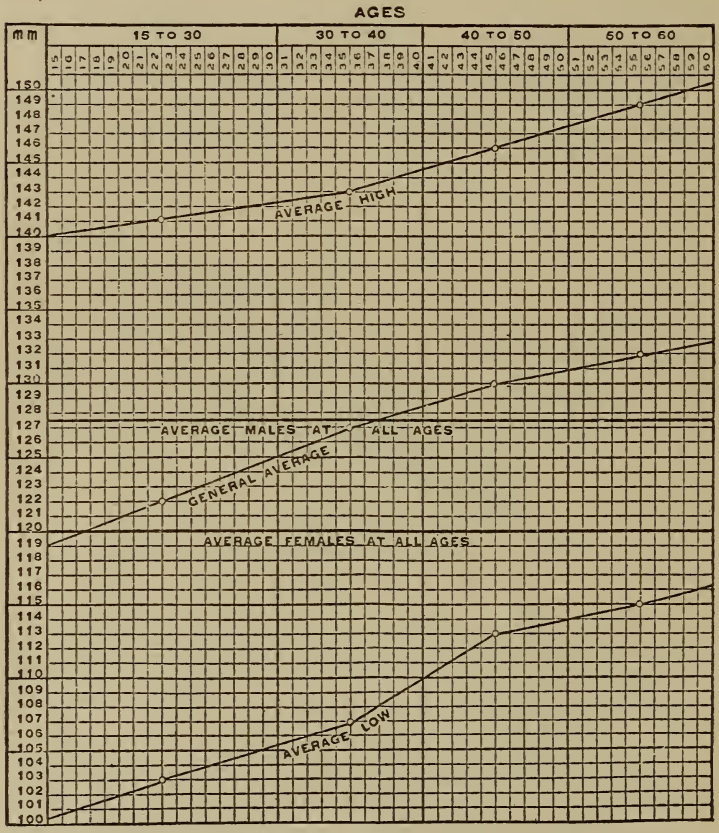


FIG. 27.—WOLEY'S CHART SHOWING EFFECT OF AGE ON BLOOD-PRESSURE GIVING MEAN, HIGH AND LOW SYSTOLIC AVERAGE.

contribution to our knowledge of normal blood-pressure, the results of which are shown in the accompanying chart (Fig. 27). Except for slight variation, the figures obtained by this observer are in accord with the results given by our best authorities.

[5] Jour. Amer. Med. Assoc., 1910, vol. lv, No. 2, p. 121.

In children under 2 years of age, Cook places the normal systolic pressure between 75 and 90 millimeters Hg. According to Lauder Brunton,[6] the maximum pressure in children between 8 and 14 years is 90 millimeters Hg; in youths from 15 to 21 years, from 100 to 120 millimeters Hg.

As early adult life is passed we have to deal with those normal changes in the cardio-vascular-renal system which are the result of the wear and tear of every-day life, and which leave their mark in a lessened arterial elasticity, a lessened functional activity of the kidneys, and by inducing degenerative changes in the myocardium often eventuate in chronic myocarditis. The inevitable result of these changes is a gradual elevation in the normal systolic blood-pressure. We have now to establish new normals by which we may determine the pathologic. For this purpose I have devised and employed the following rule: Consider the normal average systolic blood-pressure at age 20 to be 120 millimeters Hg, then for each year of life above this add one-half of 1 millimeter to 120. This at the age of 60 means an average systolic pressure of 145 millimeters Hg, which coincides closely with the figure given by Woley, Janeway, and others.

Size and Temperament.—With the standard armlet the factor of size of the individual does not enter. Temperament, on the other hand, does undoubtedly affect the reading, because in the nervous it is often impossible to remove entirely the effect of psychic influence; therefore, allowance must be made for an of psychic influence; therefore, allowance must be made for an ing degree in a limited period of time.

Diurnal Influence.—A record of blood-pressure taken at frequent intervals during twenty-four hours makes a very striking picture. The influence of this factor is most difficult to estimate. Janeway suggests a variation of 60 millimeters Hg as the extreme. I have not seen it equal this. Authorities agree that the lowest blood-pressure is reached during the early hours of sleep and that a gradual rise occurs toward morning.[7] During the day there is a physiologic rise which reaches the maximum in the evening.

Posture.—This should not be confounded with the effect

[6] Lancet, Oct. 17, 1898.
[7] Brush and Fairweather: Amer. Jour. Physiol., vol. v, p. 199.

of gravity, which may be eliminated by making all observations with the cuff at the level of the heart. Authorities differ; Janeway, and Erlanger and Hooker hold that the effect of posture is insignificant, while O. Z. Stephens[8] and A. M. Sanford[9] demonstrate by the examination of a large number of normal individuals that this has a very constant and definite influence.

Their observations, with which mine agree, show that there is little alteration in pressure between the standing and sitting postures; occasionally there is a rise of a few millimeters. Between the standing and the recumbent Stephens reports an elevation often amounting to 20 millimeters Hg, while Sanford notes a rise amounting to only half of this. Between the standing and the head down (Trendelenburg) the rise in pressure may reach 35 millimeters Hg. These observers note a compensatory lowering of pulse rate, upon which Schapiro has based a test for the functional capacity of the heart.

Prolonged rest in bed by one accustomed to active exercise, especially if there was a tendency to high pressure, causes a rapid and marked fall with the establishment of a new systolic level.

Emotion and Excitement, Including Pain.—In determining the influence of psychic disturbances, temperament plays an important part. The pressure-raising effect of pain, fright, fear, and apprehension must always be recognized. Vasomotor disturbances from sensations of heat or cold and changes in the arm from prolonged pressure of the arm-band must not be ignored. Every effort should be made during the test to eliminate psychic and vasomotor disturbances by establishing a proper understanding between the patient and physician, and by completing the test with as little delay as possible.

Exercise.—Muscular exertion in the healthy, especially if sharp, may cause an elevation in blood-pressure of from 5 to 15 millimeters. This elevation becomes less marked as the subject becomes accustomed to performing the act or acts. This reduction in susceptibility of the cardiovascular system is one of the beneficial effects of training.

When effort is prolonged but moderate in severity (as in

[8] Jour. Amer. Med. Assoc., Oct. 1, 1904.
[9] *Ibid.*, Feb. 18, 1898.

walking) the systolic pressure may rise from 5 to 10 millimeters, but soon adjusts itself to a new level upon which additional exertion has little if any effect, until a condition of fatigue is reached. Fatigue from prolonged exertion results in a fall in pressure, which increases until a dangerously low pressure is reached, if the effort is continued.[10] During moderate exercise in a normal healthy person the systolic and diastolic pressures tend to become more widely separated, *i.e.*, the pulse-pressure becomes greater.[11] Upon this physiologic fact is based the work test of Graüpner.[12]

Passive movements and massage, except when causing pressure upon the thorax or abdomen, can be prolonged without materially altering the systolic pressure (Eichberg).

Diet and Digestion.—The difficulties in the way of accurately gauging the effect of diet and digestion on blood-pressure are great, because of the difficulty met in eliminating conditions bordering on the pathologic. The effect of overeating, overingestion of fluids, including alcohol, insufficient exercise and defective elimination must all be considered and weighed. Observers differ upon this question, some holding that as a result of the normal splanchnic engorgement blood-pressure falls a few millimeters after eating, while others affirm that observation shows a normal rise in pressure amounting to from 10 to 20 millimeters, and that the rise may exceed this when large quantities of fluid, particularly beer, are taken with the meal.

Alcohol.—Clinical evidence so far shows that a moderate amount of alcoholic beverages taken regularly does not materially influence blood-pressure. The irregular use of alcohol occasions first a rise of from 10 to 20 millimeters followed shortly by a fall as the superficial capillaries become dilated.

Tobacco.—Little has been added to our knowledge of the effect of smoking on blood-pressure since the contribution of H. A. Hare which appeared about twenty years ago. We find that the moderate use of tobacco in those accustomed to its habitual use causes at first a sedative action with lowering of blood-pressure, while if used immoderately the pressure is elevated. A long series of tests by the writer, upon three healthy subjects, showed

[10] Karrington: Zeitschr. f. klin. Med., 1903, vol. L, p. 322.
[11] Krehl: "Clinical Pathology," 1905, 3d ed.
[12] "Die Messung. der Herzkraft," 1905.

a fall in blood-pressure from 4 to 10 millimeters after smoking one cigar. If this was followed by a second, the pressure tended to return to the original level, while after from three to five cigars smoked in succession the pressure rose from 2 to 15 millimeters above the original reading.

Pathologic Variations in Blood-pressure.—For convenience in study we may appropriately divide pathologic alterations in blood-pressure into pathologic high pressure and pathologic low pressure.

Pathologic High Blood-pressure.—High pressure *per se* is not a disease, but a phenomenon or symptom, which may accompany a great variety of diseased conditions, including arteriosclerosis, angina pectoris (usually), acute nephritis, chronic interstitial nephritis, chronic parenchymatous nephritis, certain forms of valvular disease, acute endocarditis, chronic myocarditis, eclampsia, cerebral hemorrhage, arterial thrombosis, migraine (usually), lead poisoning, asphyxia, syphilis of heart and arteries.

Pathologic Low Pressure.—A pathologic depression in blood-pressure may be caused by the depressing influence of circulating toxins acting either upon the heart, blood-vessels or controlling nervous mechanism or to sudden withdrawal of a large volume of blood from the circulation as in hemorrhage, after *venesection, copious diaphoresis, diarrhea,* or in *shock.*

The lowest blood-pressure compatible with life has been reported by Neu to be from 40 to 45 millimeters of mercury, and this only occurred with subnormal temperature in the moribund state. He has seen recovery after a fall in pressure down to 50 millimeters.

It is noted that a moderate and progressively falling pressure occurs in most progressive and prolonged fevers, as in typhoid fever. When due to such a cause the depression is rapidly overcome and disappears as convalescence is established.

Widespread dilatation of the vessels and consequent lowering of blood-pressure have been noted in the last stages of *arteriosclerosis* (Krehl).

Functional Tests in Chronic Myocarditis.—The sphygmomanometer is a most valuable means of detecting alterations

in the musculature of the heart, often before the development of the usual physical signs.

In the physical examination the state of the superficial vessels, together with the pulse-rate and particularly the reaction of the heart to posture and exercise as determined by the sphygmomanometer, is all-important. This latter may be determined by the following tests:—

Work Test.—Moderate exertion raises pressure in normal hearts and this rise is sustained during it if not unduly severe or prolonged. In weakened heart muscle from any cause a primary rise may occur, but is quickly followed by a fall; in the most serious a fall occurs from the first.

Graüpner's Test.[13]—This is based upon the physiologic fact that a given amount of exercise, such as ten bending movements or running up a flight of stairs, causes an acceleration in the pulse-rate and an elevation in blood-pressure. But the latter does not appear coincidently with the former; or, if, as in some cases, the pressure does rise first, it fails to rise again after the pulse has returned to normal. It is this secondary rise which indicated a good heart muscle. A not too seriously affected heart may show a rise in blood-pressure immediately after the exertion, but with the slowing of the pulse the pressure will be found to have fallen to a level lower than before the experiment. The sphygmomanometer is required for an accurate demonstration of these changes in pressure, which may be recorded in definite units of measure for future reference and comparison.

Schapiro's Test.—This is based upon the alteration in pulse-rate occurring in normal individuals on change of posture from the standing to the recumbent. Normally, the number of pulse beats per minute is from seven to ten less in the recumbent position; but when chronic myocarditis develops this difference tends to disappear, so that in seriously weakened hearts the pulse may be as rapid in the recumbent as in the sitting posture.

Cautions.—*It is not advisable to apply this test to patients with excessively high blood-pressure, in those of apoplectic tendency, or in those with high-grade arteriosclerosis.* The test is unsafe in those with a systolic pressure of 200 millimeters or

[13] Berlin. clin. Wochen., xliv, 1907, No. 15.

over. In such cases there is danger of ocular or cerebral hemorrhage or acute dilatation of heart.

The test will be difficult if not impossible of application in women unless all tight clothing is removed.

Valvular disease is not necessarily a contraindication to this test, as the condition of the myocardium seems to be the only important factor, except in aortic regurgitation with high pressure, so that the presence of valvular lesions need not detract from the value of the information obtained by this test.

In **life-insurance examinations**[14] it is now well recognized that such pathologic changes may be present in the cardiovascular and renal systems long before any suggestive symptoms are complained of by the individual, or if any complaint is made it is usually attributed to some trivial cause.

In the presence of such a beginning arteriosclerosis the blood-pressure need not be greatly increased; an elevation of 30 to 40 millimeters above that estimated as normal for the individual is significant and demands explanation. On the other hand, a rise of even this amount should never be hastily assigned to arteriosclerosis and the risk therefore rejected without further study.

It is necessary to recognize in this connection the activity of other and less important factors, such as the alimentary hypertension, so well described by Russell, occurring in normal vessels due to errors in diet of either quantitative or qualitative origin and responding immediately to the correction of such errors, together with stimulation of the eliminative functions. Of further interest, particularly to the life-insurance examiner, are the so-called physiologic variations, caused by age, sex, mental and physical excitement, fatigue, etc. These must all be taken into consideration in estimating the character and class of the risk. (See page 113.)

Such variations need not confuse the examiner, as they all occur within a range sufficiently restricted to prevent them from obscuring the issue. The only one which needs special consideration is the age factor. To determine this, employ the author's formula explained on page 115.

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14 "The Insurance Examiner and the Blood-pressure Test," F. A. Faught, Medical Record, Aug. 10, 1912.

High Pressure and Transient Albuminuria.—Probably the most confusing combination of symptoms met is the case which presents a slight hypertension and an occasional trace of albumin in the urine. These cases are best examined at the home or branch office and should be referred there whenever possible, for it is only after very careful and complete examination with repeated urine and blood-pressure tests that a correct conclusion regarding the safety of the applicant can be reached.

If after eliminating the possibility of an alimentary hypertension a distinct elevation in pressure remains with albumin in the urine, even in occasional minute traces, the risk is doubtfully good, while if accomplished by accentuation of the aortic second sound or casts the risk is bad and calls for rejection.

B. SPHYGMOGRAPHY.

Sphygmography is the method of registering the pulse wave in some peripheral vessel, generally the radial artery, upon a moving surface (usually smoked paper) by means of a special instrument, the sphygmograph.

The apparatuses which are employed for this study are, even the simplest of them, complicated and difficult to manage. The essence of the method should be simplicity, for the more complicated the procedure, the more unsuitable it becomes for practical clinical purposes. This requirement does not obtain in hospitals with large staffs and much clinical assistance.

The Sphygmograph.—It is scarcely necessary to enter into a full account of the construction of the various sphygmographs. They are practically all constructed on the same principle. A steel spring is laid upon the radial artery at the wrist in such a manner that, while it compresses the artery, it does not obliterate it. Attached directly to the spring is a long lever, or a series of small levers, that magnify the movements of the spring. The free extremity of the lever presses lightly against a strip of paper whose surface has been blackened by the smoke of burning camphor or turpentine, the strip of paper passing at a uniform speed by means of a clockwork arrangement. The simplest and most practical sphygmograph is that of Dudgeon

(Fig. 28), which, after a little practice, may be relied upon to give a true and accurate record of the pulse wave.

The Polygraph (Fig. 29).—There are many perceptible movements due to the circulation that the sphygmograph fails to register, and when it is desired to record these movements



FIG. 28.—DUDGEON'S SPHYGMOGRAPH.

other instruments have to be used. The method most commonly employed has been to convey, by means of a tubular air system, the movements to be registered, to a recording tambour operating against a suitable recording surface. Two tambours are usually employed, with their levers placed one above the other, so that simultaneous records of these different movements, the apex beat and jugular pulse, can be readily reproduced.

Jaquet's polygraph consists of a metal frame to which is screwed a cuff for attaching to the wrist. The sphygmograph proper is attached to the frame. The window cut out of the

frame is to be applied accurately along the radial artery (previously marked out with a pencil), and the cuff then strapped around the wrist quite tightly. The sphygmograph proper is set into the frame by hooking into the hinge, and then the connection of the two parts is effected by pressing down at and tightening the screw. The pulse-registering apparatus consists of a short, broad spring, which presses upon the artery and transmits

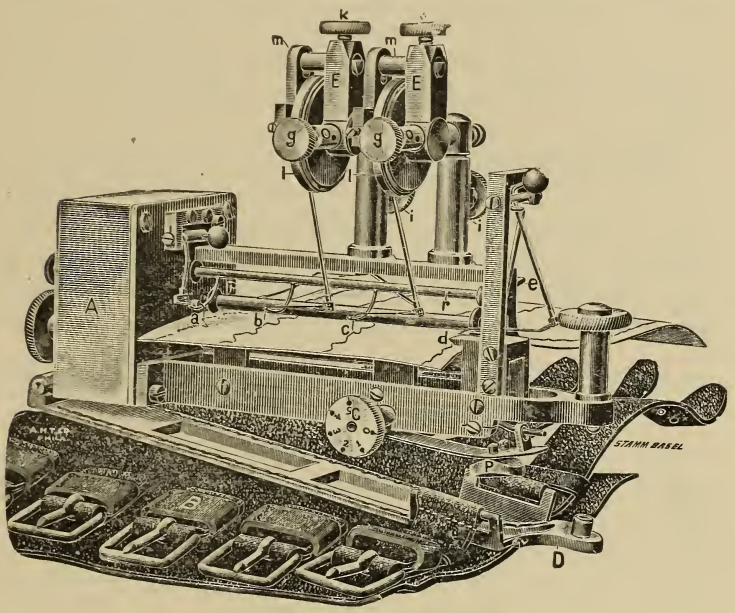


Fig. 29.—Jaquet Polygraph.

its movements to the registering-needle by means of the lever system. The screw also serves to adjust the registering-needle at the desired height upon the smoked strip of paper. By screwing it down, the spring is pressed against the artery. The screw is connected with an "eccentric" contrived to increase or diminish the pressure upon the spring. The amount of pressure can be determined by noting the position of the figures upon the screw. With this mechanism the instrument can be adjusted with practically equal pressure in each case, and taken away from the frame and reapplied in the same case, without alteration of the pressure. The paper is run through in a horizontal position and

it may be made of any convenient length. A little box contains the clockwork which moves the strip of paper, which is controlled by pressing down on the lever. The rate that the paper moves may be increased from 1 to 4 centimeters in a second by altering the position of the lever. The slower motion produces a curve which presents a better general idea; the more rapid motion, one which may be more accurately analyzed. This rate motion may be altered while the sphygmograph is in action. The box also contains a watch actuating a time-registering mechanism. The latter consists of a small stylus which registers a mark upon the margin of the smoked ribbon every one-fifth second.

The Ink Polygraph.—This simple and useful device has been perfected by MacKenzie; it records in ink on a roll of paper, which can be of any desired length, at the same time obviating the inconvenience of blackening and varnishing the paper. In operation this instrument is very similar to the one just described.

EXPLANATION OF A NORMAL PULSE CURVE; FACTORS WHICH INFLUENCE ITS FORM.

The curves obtained with the good modern sphygmographs usually correspond quite uniformly. The pulse wave is composed of a steep ascending and a rather slanting descending limb, in which the sphygmographic tracings show a number of small elevations in the descending limb (so-called catacrotic elevations). Similar irregularities which may appear under pathologic conditions in the ascending limb are termed anacrotic elevations. A pulse with catacrotic elevations is termed catacrotic; one with anacrotic elevations, anacrotic.

Generally speaking, the curve varies under the following circumstances (MacKenzie):—

1. Other things being equal, the curve is lower the higher the mean blood-pressure, and *vice versâ*. This can be understood easily, because with a high blood-pressure the arterial wall is already so tense that even during diastole the increase in pressure during systole can produce but a very small excursion. When, on the contrary, the pressure is low, the artery easily yields to the change. Of course, this does not apply to those

cases where the high pressure is produced by a large systole (left-sided cardiac dilatation and hypertrophy in compensated heart affections), nor where a low pressure is due to a small systole (disturbances of compensation).

2. Other things being equal (the same blood-pressure), the pulse curve is higher the larger the systole, and *vice versâ*.

3. Other things being equal (equal systole and equal blood-pressure), the ascending limb of the curve is steeper the quicker systole takes place.

4. Other things being equal, a low mean blood-pressure produces both a steep ascent and a steep descent, *i.e.*, a pointed curve (celerity of curve *in toto*). Conversely, a high blood-pressure produces a slanting rise and a gradual descent (tardiness of curve).

5. Other things being equal, rigid arterial walls (like high blood-pressure) produce low curves with slanting ascent and gradual descent (tardiness). On the contrary, delicate elastic arteries (like low blood-pressure) produce curves with steep ascents and descents (celerity).

V.

ANIMAL PARASITES.

PARASITES IN THE BLOOD.

THE PLASMODIUM OF MALARIA.

This is the only sporozoa found in the blood which is connected with disease in man, although numerous hemosporidia have been reported in many of the lower animals.

History of the Malarial Parasite.—This is one of the most interesting chapters in medicine. The parasite was discovered by Laveran in 1880, but it was not until 1885 that Golgi observed that sporulation occurred simultaneously with the malarial paroxysm. Golgi also demonstrated the existence of different species for different types of fever.

Life History.—When man is first infected there commences a non-sexual cycle which is completed in forty-eight or seventy-two hours, depending upon the species of parasite. The *sporozoite* bores into a red cell, assumes a spherical form, and continues to enlarge. As it approaches maturity, it shows signs of division into a varying number of spore-like bodies. The parasite at this stage is termed a *merocyte.* When the merocyte ruptures, these spore-like bodies, or sporozoites, each enter a fresh red cell and develop as before. At the time these merocytes rupture, it is believed that a toxin is liberated which causes the malarial paroxysm. The cycle goes on by geometric progression. From the first indication of the sporozoite it is usually two weeks before a sufficient number of merocytes rupture simultaneously to produce toxic symptoms (the period of incubation). This cycle is termed *schizogony.* After a varying time sexual forms develop. These are termed *gametes,* and show two types, one which contains more pigment, has little chromatin, and stains more deeply; this is the female, or *macrogamete;* in the other there is little pigment, much more chromatin, and it stains less deeply; this is the male, or *microgametocyte.*

DESCRIPTION OF PLATE IV

Malarial Parasites. (*Kolle & Wassermann.*)

1. Two tertian parasites about thirty-six hours old, attacked blood-corpuscles magnified.

2. Tertian parasite about thirty-six hours old; stained by Romanowsky's method. The black granule in the parasite is not pigment but chromatin. Next to it and to the left is a large lymphocyte, and under it the black spot is a blood-plate.

3. Tertian parasite, division form nearby is a polynuclear leukocyte.

4. Quartan parasite, ribbon form.

5. Quartan parasite, undergoing division.

6. Tropical fever parasite (Æstivo-autumnal.). In one blood-corpuscle may be seen a smaller, medium, and large tropical fever-ring parasite.

7. Tropical fever parasite. Gametes half-moon spherical form. Smear from bone marrow.

8. Tropical fever parasite, which is preparing for division heaped up in the blood capillaries of the brain.

Asexual Forms

9. Smaller tertian ring about twelve hours old.

10. Tertian parasite about thirty-six hours old, so-called ameboid form.

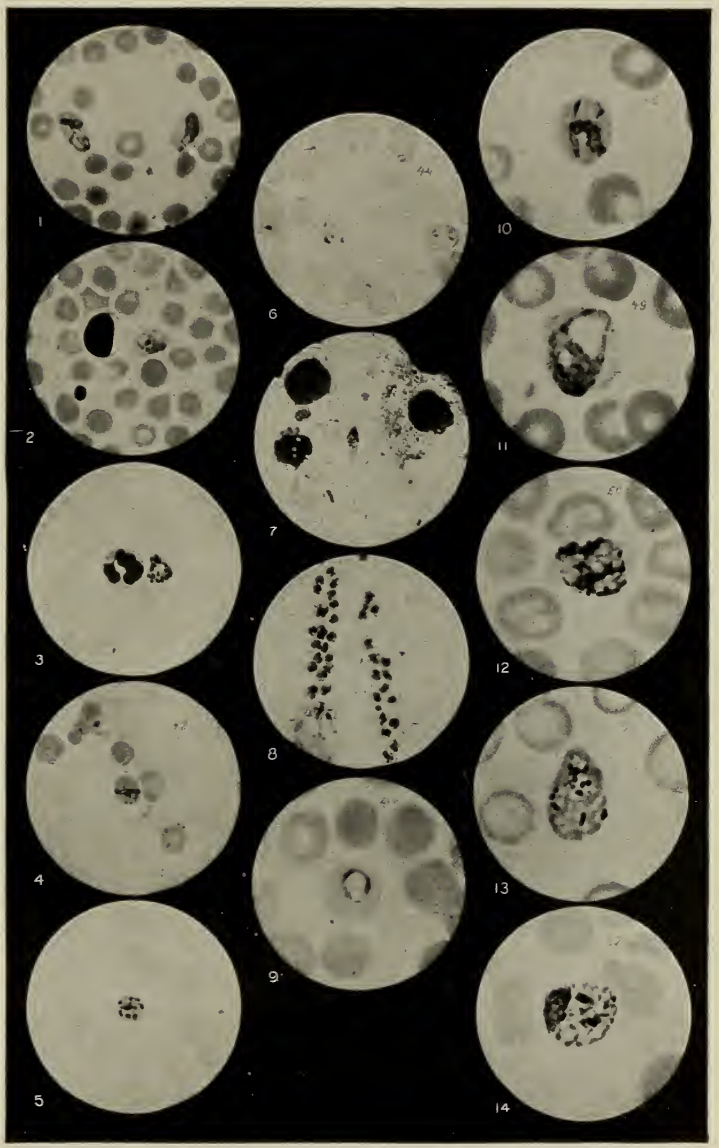
11. Tertian parasite still showing ring fever, forty-two hours old.

12. Tertian parasite, two hours before febrile attack. The pigment is beginning to arrange itself in streaks or lines.

13. Tertian parasite further advanced in division. Pigment collected in large quantities.

14. Further advanced in the division. (Tertian parasite.)

PLATE IV



When the gametes are taken into the stomach of the mosquito (Anophelinæ), the male cell shows tail-like projections which have an active lashing movement, which break off from the cell carrier and are thereafter termed *microgametes.* These enter the macrogametes and the combination forms a *zygote.* The zygote enters the epithelial layer of the stomach of the mosquito, where it continues to enlarge for about one week. When it has reached the size of about 60 microns it is seen to contain hundreds of delicate falciform bodies. The mature zygote now ruptures, and the sporozoites are thrown off into the midgut of the mosquito, whence they make their way to the salivary glands, from which they are introduced into the circulation of the person bitten by the mosquito, and then start the non-sexual cycle already described. As the sexual cycle takes place into the mosquito, this insect is the definitive host, while man is the intermediary host.

There are three species of malarial parasites: first, *P. vivax,* or benign tertian, with a cycle of forty-eight hours; second, the *P. malariæ,* or quartan, with a cycle of seventy-two hours, and, third, the *P. falciform,* or estivo-autumnal or malignant tertian, with a cycle of forty-eight hours.

Variations in cycle may be produced by infections from mosquitoes on successive nights, so that one will mature and sporulate twenty-four hours before the second. This will give a quotidian type of fever. In the estivo-autumnal infections acceleration or retardation in sporulation will cause a very protracted paroxysm lasting from eighteen to thirty-six hours. This tends to give a continued fever instead of the characteristic type.

In the diagnosis of malaria one should also examine both the fresh and the stained specimen, as each gives valuable information in differentiating species. When time will not permit the examination by both methods, always use the smear stained by Leishmann's stain, as the small, externally situated rings of estivo-autumnal fever may escape notice in a fresh specimen.

It is evident that, while there is considerable similarity among the three varieties of parasites, there are, nevertheless,

certain peculiarities and characteristics which enable the expert to quite positively differentiate between them.

DIFFERENTIAL DIAGNOSIS OF PLASMODIA.

TERTIAN.	QUARTAN.	ESTIVO-AUTUMNAL.
Develops in 48 hours.	Develops in 72 hours.	Develops in 24 to 48 hours.
Pale and indistinct.	Sharp outline; refractile.	Has irregular appearance.
Actively ameboid.	Slightly ameboid.	Actively ameboid.
Pigment fine.	Pigment coarse.	Pigment granules very few.
Pigment actively motile.	Pigment slow in movement.	Pigment still.
Pigment light.	Pigment dark.	Pigment light.
Full size of corpuscle.	Smaller than corpuscle.	Smaller than corpuscle.
Degeneration forms twice the size of corpuscle.	Degeneration forms vary; much smaller than tertian.	Absent.
Segments 16 to 20.	Segments 5 to 12.	Segmentation forms not seen in peripheral blood.
Irregular segments are frequent.	Beautiful rosettes.	
Corpuscles large, colorless, and swollen.	Corpuscles shrunken and brassy.	Corpuscles shriveled, brassy, not decolorized.
		Forms crescents.

THE DETECTION OF THE PLASMODIUM.

In searching for the organism it is always desirable, when possible, to examine the fresh specimen at the bedside of the patient. If this is impracticable, warm the slide and seal the cover with a little vaselin, and the organisms will retain their motility for at least a couple of hours.

If considerable time must elapse before the examination, films should be made and dried preparatory to staining, which can then be done at a convenient time.

Staining Methods.—Do not have the blood-films too thick; the individual cells should not overlap; there should be no rouleau formation. Allow the films to air-dry without heat and then, without previous fixing, stain with one of the polychrome stains. Wright's is very satisfactory (see Appendix for stain). Do not give up the search until all the films have been examined thoroughly. A number of fields may show no organisms; then some may appear. The specimen is best obtained either eight or ten hours before or after a chill, as at this time the organisms

are most likely to be in the peripheral circulation. Medication should be withheld for as long a time before the specimen is taken as possible.

What to Examine For.—Fresh specimen: Look for red cells containing actively moving black specks (pigment granules and living protoplasm). Unusually pale cells containing clear areas which are irregular and constantly changing shape. Extra large red blood-cells.

After a little experience the pigmented organisms are readily distinguished. Violent commotion among a group of red cells will direct attention to the flagellate. The hyaline bodies are usually the most difficult to identify without experience. The apparent, but not real (artefact), may be found in large numbers; these are usually distorted and deformed cells that have become altered in the preparation of the specimen. Finally, pigment-bearing leukocytes may be found in excess in malaria. These are, as a rule, polymorphonuclears which have become phagocytic, and have taken up the iron pigment set free by the malarial organisms.

Examination of the Stained Specimen.—Unless one has had some experience, the stained specimen will yield much more definite results than the fresh, provided that the staining technic is good. The slight refractility, both of the cell and of the parasite, in the fresh specimen makes it difficult to get the proper illumination. In the hands of an expert the examination of fresh blood is usually all that is required for a diagnosis in the average case, and when the parasites are moderately numerous even the beginner can scarcely make a mistake. It would seem, therefore, inadvisable to settle the diagnosis after an examination of the fresh specimen in case no organisms are found, but to control this examination by a careful study of a stained specimen, where one may frequently be surprised at the number of parasites seen.

Caution.—Frequently one observes in stained specimens many artefacts due to deposition of staining pigments upon the red cell (see above), while in the fresh specimen areas of coagulation necrosis are not infrequently seen, so that the beginner may incorrectly assume the presence of malarial organisms. For an absolute diagnosis of malaria, it is necessary to find intracel-

lular organisms, and not to be content with a single examination in doubtful cases.

CULTIVATION OF MALARIA PLASMODIA.

C. C. Bass[1] on several occasions has reported his work in the cultivation of the three species of the malarial parasite. In order to cultivate the malarial parasite, Bass recommends media containing human serum, Locke's fluid (from which the calcium chlorid is omitted), and human ascitic fluid. The addition of a percentage of dextrose facilitates the growth. The suspected blood is transferred to this medium, in which it is found that the plasmodia grow in a thin layer near the top of the cell sediment, beneath which zone the parasites all die. Parasites develop readily in the red corpuscles, but are only maintained alive while in the human red blood-cells, as they are destroyed by the leukocytes, by serum, Locke's fluid, ascitic fluid, etc., as soon as liberation occurs.

The most favorable temperature for cultivation is about 40° C. The latest report of this observer shows positive cultures obtained in 29 cases of the estivo-autumnal type, in 6 cases of tertian, and in 1 case of quartan. Cultures have been carried out as far as four generations. Only the asexual cycle has been observed. The chief advantage to be derived from the employment of this method would seem to be the fact that in the proper surroundings a small ring body will rapidly be developed in the segmentation form, thereby rendering microscopic recognition of this infection less difficult.

THE PRESENT STATUS OF THE PARASITE OF SCARLET FEVER.

Owing to the obscure origin, frequency, and seriousness of complications, many observers have directed their special efforts toward the finding of the specific cause of this disease. It has been more or less generally accepted that one or more of the strains of streptococcus were closely involved in the production of the disease and also are important determining factors in the development of many of the complications. Recently Klimenko[2]

[1] Journal A. M. A., Part 1, September 21, 1912.
[2] Centralblatt für Bakter., Bd. 65, p. 45.

published a series of exhaustive bacteriologic studies upon 523 cases of scarlet fever in an effort to determine this relation. His results are important. In only 11 cases was a streptococcus recovered from the blood during life, yet these cases were all of a complicated or serious variety. The organisms, according to this observer, seem to have no relation to kidney, heart, or joint complications even when present. From his studies he concludes that the streptococcus is never present in the blood in the early stage of scarlet fever, and that it bears no causal relation either to the disease or to its complications. We must, therefore, consider the probability that the streptococcus bears no direct relation to scarlet fever.

Dohle[3] reports finding certain bodies in the polymorphonuclears in scarlet fever. These he calls "leukocytic inclusion bodies." They are round, oval, or rod-shaped, and vary greatly in size. They stain readily with all the usual blood stains and are basophilic. They do not show any chromatin staining. He believes these to be of certain diagnostic value, as in one series they were found regularly in the blood of 30 patients with scarlet fever, but rarely in other conditions.

His observations have been in part confirmed by others.

The same observer has later described a spirochæte occurring in the leucocytes in scarlet fever, and that the "inclusion bodies" may represent portions of these, which may in turn be the cause of disease. These observations yet remain to be confirmed.

Dr. John Kilmer[4] states that he found these "inclusion bodies" in 94 per cent. of 49 cases of scarlet fever, during the first three days, but that they were rarely present after the ninth day. They were also found in the same percentage of cases of diphtheria. If these observations are confirmed, we must conclude that the diagnostic value of these bodies is limited. In serum sickness with a scarlatiniform rash their absence excludes scarlet fever, while their presence in this condition may not necessarily mean scarlet fever. They have, therefore, a more negative than positive value. The technic of examination is extremely simple, requires very little time, and should be employed as an aid to differential diagnosis.

[3] Centralblatt für Bakter., Bd. 61, p. 63.
[4] American Journal of Diseases of Children, vol. iv.

Blood-smears are made in the usual way, stained by one of the accepted methods, and examined for the presence of basophilic bodies of varying size, which are round, oval, or rod-shaped and found in the polymorphonuclears.

THE PARASITE OF YELLOW FEVER.

Aristides Argamonte,[5] doubts that the so-called Seidelin bodies are the cause of yellow fever. He states that defects in staining technic may produce similar bodies, which are probably nuclear and protoplasmic fragments. However, it is by no means settled that these bodies do not represent some stage in the development of a parasite which is the cause of yellow fever. They may be demonstrated in an ordinary blood-smear taken under ordinary precautions from a patient suffering with yellow fever, and are stained best by one of the differential stains, of which Seidelin recommends the Giemsa reagent.

FILARIASIS.

This is a condition associated with the presence of filariæ in the blood (*Filaria sanguinis hominis*). While many of these filariæ are known, the most common one is the *Filaria bancrofti* (*Filaria nocturna*) (see also page 155). For the special distinguishing characteristics larger works must be consulted.

The adult or parent organism is slender and thread-like, varying from 3 to 6 inches in length. It inhabits the lymphatics and tissues, while the embryos appear in the peripheral circulation.

Originally these embryos were supposed only to appear in the circulation toward evening, their numbers gradually rising to a maximum at about midnight and diminishing toward dawn. This rule has been found by Rivas and Smith[6] to have exceptions, as shown by findings of *F. nocturna* and *F. diurna* in the same specimen of blood. During the day they are found in the internal organs, especially the lungs. The forms appearing in the blood are practically all embryos, as the adult type lies in the lymphatics, where it may obstruct the lymph flow. The movements of these embryos is at first distinctly progressive, as seen

[5] N. Y. Med. Record, August 17, 1912, p. 288.
[6] D. Rivas and Allen J. Smith, So. Med. Jour., p. 631, 1913.

under the microscope; but they soon become motionless, appearing to attach themselves to the glass slide at their anterior end.

The obstruction in the lymph-glands may also be brought about by the eggs, which are 25 to 38 microns long by 15 broad. The embryos reach the general circulation only through the thoracic duct.

Like the malarial organisms, the filaria has an intermediate host in the mosquito, both of the culex and anopheles variety. The embryos, which are taken up by the bite of the mosquito, cast off their sheath in about one hour in the stomach of a mosquito. Some of these embryos die at this stage, but others bore actively through the intestinal wall, to the muscle, where they remain. During the next two or three days, the embryo becomes larger and its alimentary tract develops. On the seventh day the worm is about 1½ millimeters long and is perfectly formed. It now travels toward the head and takes its position in the labium, whence it enters the blood of its new host during the biting of the insect. A large number of these adult forms is necessary to cause very severe cases and many years may pass before any symptoms are manifest.

In examining the blood for filaria, it is best to take a specimen late at night and to make a very thick smear, which should be examined fresh with a low power.

Besides the ordinary anemia which may develop in such cases, we find a very striking eosinophilia, which may run from 5 to 20 per cent.

A very characteristic finding in such cases is the condition of hematochyluria followed by chyluria. This hematochyluria seems to be due to rupture of the varicose lymph-vessels of the bladder, which form a large part of the collateral circulation when the thoracic duct is occluded. Such attacks may occur for years and be separated by long intervals. Their onset is spontaneous or follows exertion and is usually associated with pain and fever. The urine shows the presence of blood, chyle (as high as 3.8 per cent. fat), and embryos (Emerson).

Many other forms of filariæ are known, but this Bancroft type seems to be the most important. While this disease occurs endemically in the tropics, it is advisable in a case showing lymph tumor, elephantiasis, and hematochyluria, especially when pain

and fever and enlarged spleen are present, to examine the blood for the *Filaria bancrofti.*

METHOD OF EXAMINATION FOR FILARIA.

Rivas and Smith[7] recommend the employment of large amounts of blood especially if drawn during the day; otherwise the embryos will not be found. An experimental study showed that under the same conditions the examinations of dried and stained specimens greatly increased the percentage of successful examinations. By any method, much depends upon the time of drawing the blood.

Acetic Acid Method.—Collect 0.1 to 1 cubic centimeter of blood from the finger in 5 to 10 cubic centimeters of a 2 per cent. acetic acid solution. After shaking and centrifuging, fresh slides are made from the sediment, and examined under the microscope. This method gives positive findings in most cases of filariasis, regardless of the hour at which the blood is collected.

Dry Stained Method.—Spread and dry a thick smear of blood. Fix in any convenient way. Hemolize with distilled water or 2 per cent. acetic acid, wash and stain with hematoxylin and eosin (for stains, see Appendix).

SLEEPING SICKNESS OR TRYPANOSOMIASIS.

This disease is very prevalent in Central and West Africa, and is due to the presence of an actively motile, fusiform flagellate known as the *Trypanosoma gambiense,* which can be found in the blood-plasma (never intracorpuscularly). It moves with a screw-like motion among the red cells, which it does not seem to disturb. This parasite, doubtless, has a sexual development. Kline has demonstrated that the *Glossina palpalis* is the true host of the *Trypanosoma gambiense,* although other insects may mechanically transmit it.[8]

T. Gambiense.—Castellani gives its dimensions as from 16 to 24 microns long, and from 2 to 5 microns broad. Anteriorly it is either pointed or rounded and along one border is an undulating membrane, which is a thickening of the ectoplasm. This is continued posteriorly into a free flagellum (see C, Plate

[7] *Loc. cit.*
[8] Gould and Pyle, 1912.

V). The origin of the undulating membrane takes place from a minute spot of nuclear material of oval shape, called the kinetonucleus, situated near the anterior end of the trypanosome. About the middle of the body of the parasite is an oval mass of nuclear material, irregularly shaped, called the trophonucleus. A few chromatin granules may be seen posterior to the trophonucleus, and the cytoplasm is continued in a narrow, diminishing band extending for some distance along the flagellum. In Castellani's description, in the "Reports of the Royal Society on Sleeping Sickness," are pictures showing, besides the ordinary forms, multinucleate, polyflagellate, and non-flagellate forms.

Occurrence of Parasites in Man.—The majority of observers believe that the parasite is found in the peripheral circulation and lymph-glands during the early stages (the so-called trypanosomatic fever), and that the invasion of the cerebrospinal fluid marks the beginning of the later stages.

These parasites vary much in number, sometimes being absent from the peripheral circulation for a long period and then suddenly reappearing in large numbers. Symptoms of the disease seem to bear but little relation to the number of parasites in the peripheral blood, so that in some cases it may be necessary to examine the fluid in the edematous areas or even to puncture the cervical lymph-glands.

Methods of Isolating.—Various methods have been devised for demonstrating the trypanosome in the blood of man. Unless the trypanosomes are quite numerous they may be overlooked in the ordinary fresh blood preparations. The use of a large hanging drop, and a search of from ten to fifteen minutes, will give positive results in a larger percentage of cases. After mounting, the trypanosome should be located first with the low power of the microscope by the movements of the red blood-cells as they are lashed by the flagellum of the parasite. Further investigation should then be made with the high power. The surest method of demonstrating this organism in fresh preparations is by the use of the centrifuge. About 10 cubic centimeters of blood or cerebrospinal fluid are drawn in the usual manner into a 1 per cent. sodium citrate solution or into normal salt solution,

to prevent coagulation, centrifuged and diluted three times, and the third or fourth residue examined.

Methods of Staining.—For staining blood preparations the various modifications of the Romanowsky stains give very good results. Koch recommends the use of a heavy drop of blood, stained with a dilute solution of Giemsa's stain. In the hands of experts this is a very reliable method, but one or two trypanosomes may be overlooked on account of their being concealed by red blood-cells and fibrin.

When these parasites are stained with a polychrome dye they show a rather large nucleus about the middle, a centrosome staining intensely in a vacuole-like area near the blunt posterior end, and a line of chromatin, making a dense red stain, running down the edge of the undulating membrane, and terminating in the flagellum, which is also stained red. The protoplasm of the body takes a distinct blue stain.

The parasite contains no pigment and, therefore, obtains its nourishment from the plasma and not from the red cell.

In appearance *Glossina palpalis* is a dark-colored fly, about 8 to 12 millimeters long. A point of recognition is the arrangement of the fly's wings in the resting position. They overlap like a pair of scissors. This point differentiates the tsetse fly from the other blood-sucking diptera with which it is associated.

There are many other types of trypanosomata, but the gambiense form is the more important. This is pathogenic for man, but cannot be distinguished from the trypanosoma of the tsetse fly, which is so fatal to the horse and mule (*Trypanosoma Brucei*), that of the surra disease (*Trypanosoma Evansi*), or that of dourine (*Trypanosoma equiperdum*).

RELAPSING FEVER (FERRIS RECURRENS; SPIRILLUM FEVER; FAMINE FEVER; SEVEN-DAY FEVER; TYPHUS ICTEROIDES).

The Organism.—The specific cause of relapsing fever is the spirochæta (spirillum) Obermeieri, formerly regarded as a bacterium of the genus spirochæta, but now regarded as probably a protozoan parasite—a trypanosome. First discovered by Obermeier in the blood of persons ill with the disease, it is known by his name. It is a narrow spiral about 0.025 to 0.05 millimeters

(16 to 40 microns) in length; that is, its length is from 3 to 6 times the width of a red blood disc and about 1 micron in width. It is thin, sharply curved, and appears to be structureless.

The Fresh Specimen.—Blood may be prepared after the same manner as that employed in searching for the malarial parasite and should first be examined by the medium power in order to more easily locate the parasites.

It is seen in the blood only during the febrile period of the disease, and at that time is actively motile, with a rapid, wavy motion, much resembling the movements of a coiled spring in its stretching and collapsing. It moves rather slowly among the corpuscles, but does not disturb them to any extent. Before the crisis and in the intervals the organism is not found; but small, glistening spherules, said to be its spores, take its place. Confirmation of the contagious nature of the disease is found in the fact that it has been communicated from one human being to another by inoculation of blood, and to monkeys in the same way.

Staining Method.—Smears of blood taken at the proper time (during the febrile period) are made and dried in the usual manner, after which they should be stained by one of the simple stains.

The organism takes a deep chromatin stain and also stains with methylene-blue in from two to five minutes.

Loewenthal has applied the agglutination test to the blood of suspected cases and found the reaction positive in 85 per cent. of the cases during the periods in which the parasites are absent.

Pathologic Change.—There is no essential morbid anatomy, and such as is found corresponds with that of typhus. Most conspicuous is enlargement of the spleen.

The changes in the blood are not characteristic of this condition. The red blood-cells seem to be slightly diminished in number for several days after the beginning of the attack, but rapidly return to normal during the afebrile period. The hemoglobin may be reduced to as low as 50 per cent., so that a very distinct anemia of the secondary type is common. The leukocytes seem to be distinctly increased in this disease, the most marked leukocytosis occurring just after the crisis, which subsides to, or nearly to, normal, during the afebrile period of the disease.

KALA-AZAR (TROPICAL SPLENOMEGALY; CACHEXIAL FEVER; DUM-DUM FEVER).

Through the researches of Donovan, Leishman, and Ross, parasites have been demonstrated in the blood which are probably directly associated with the condition known as kala-azar. The organism is usually known as the Leishman-Donovan body, and is a small, oval, round, or oat-shaped body from 2 to 3 microns in diameter. These bodies have a definite cell outline and contain two chromatin masses. The larger one, or nucleus, is almost round or oval and stains faintly, while the smaller, bacillus-shaped centrosome stains deeply and is directed almost at right angles to the axis of the nucleus. These two chromatin masses are both in the long axis of the cell, and the outline of the cell cannot always be seen, although these two masses thus arranged are distinctive. These bodies probably represent a stage in the development of a trypanosome, as shown by the work of Leishmann and Statham. They are not found in the circulating blood, as a rule, but they have occasionally been reported in the form of intracellular bodies in fatal cases.

Occurrence of Bodies.—The bodies may be found in smears made from blood obtained by splenic puncture and in the granulation tissue taken from the ulcers; also in the mesenteric lymph-glands, the bone-marrow, and the liver. Some of these bodies lie free, but most of them are intracellular, either in the leukocytes, endothelial or splenic cells, and frequently in large masses in the macrophages.

Method of Staining.—The films should be thinly spread, after proper fixation, or without, if a form of Romanowsky stain be used, and then stained in the usual way. By Wright's method the chromatin appears dark, the cell-body blue, and the remainder a fainter mauve.

Blood Changes.—The changes in the blood are those of a moderate anemia, associated with a leukopenia and a relative and absolute increase in the number of the large mononuclears. The average leukocyte count is about 2000.

SYPHILIS.

The *Spirochæta pallida* (*Treponema pallidum*) derives its name from its low refractive power and the difficulty with which

it takes aniline dyes. It is a very delicate structure, presenting 10 to 40 deep spiral incurvations in the larger specimens or only a few in the smaller ones. Its length varies between 4 and 10 microns and its width does not exceed ½ micron. The organism has been demonstrated in the circulating blood, in the scrapings obtained from the chancre, in incised papules, in smears from the mucous patches, and in fluid aspirated from the inguinal glands. It seems to be easily demonstrable in the blood from a splenic puncture, while in the congenital forms it is found in the internal organs and in the peripheral blood. A characteristic difference between this spirochæta and some other types with which it might be confused is that its ends lie above and below a longitudinal line drawn through the center of its curvatures, while in the other forms the ends lie on the projection of such a line. The organism moves in an oscillatory manner about its longitudinal axis, its movements being winding, bending, and whipping. Schaudinn demonstrated the existence of a flagellum at each end, while the other spirochætes have an undulating membrane.

It is in active motion when examined in the fresh, and may maintain its activity for days in salt solution, when the tissues are kept at 20° to 27° C. (Beer).

Methods of Obtaining a Specimen for Examination.—The surface from which the specimen is to be taken is cleansed with alcohol, irrigated with salt solution, and dried. It is then scraped with a needle, care being taken to avoid drawing blood, but bringing out a good deal of serum. Smears are made from the serum, as it is in this that the spirochæte will be found. If desired, serum may be drawn from an enlarged gland by means of a hypodermic needle and the smears made from this.

STAINING METHODS.

Giemsa Method.—The smear is dried in the air, then placed for an hour in absolute alcohol, and then stained for twenty-four hours in diluted Giemsa mixture. (For preparation of stain see Appendix, page 409.) One drop of Giemsa stain to 1 cubic centimeter of the distilled water is the strength used. The organism stains a delicate violet-purple color, while the nuclei of the leukocytes are of a deep blackish red. The time for stain-

ing may be materially shortened by adding a few drops of a $\frac{1}{1000}$ solution of potassium carbonate to the diluting water. By this expedient the spirochæte may be demonstrated in fifteen minutes, though for the best results prolonged contact with the stain is advised.

Method of Oppenheim and Sachs.—The smears are dried in the air and then, without previous fixation, are flooded with an alcoholic solution of carbol-gentian-violet (for preparation of stain see Appendix). After flooding the preparation it is warmed until it steams for a few minutes only; it is then washed gently in running water, dried, and examined. The spirochætes are stained blue.

Goldhorn Method.—The smears are fixed with pure methyl alcohol for fifteen minutes and are then covered with the stain (polychrome methylene-blue) for three to five seconds, when the excess is drained off. The specimens are then slowly introduced into clean water with the film sides down. Keep the slides in this position for four or five seconds and then shake in the water to remove the excess of the dye. The spirochætes appear of a violet color. This violet tint may be changed to a bluish black by covering the specimen with Gram's iodine solution for from fifteen to twenty seconds, after which it is washed and dried as usual and the examination made with the immersion lens.

Method of F. C. Wood[9] (fifteen minutes).—The smears are fixed in strong methyl alcohol and dried with blotting paper. The following stains are then applied in succession:—

First, a few drops of a $\frac{1}{1000}$ watery solution of yellow water soluble eosin are spread over the smear by means of a pipette. Second, four or five drops of a $\frac{3}{1000}$ aqueous solution of methylene azure II. The two colors are thoroughly mixed by spreading over the smear and by rocking the cover-glass. This mixture is allowed to act for eight minutes. The stain is then washed off by a strong stream of distilled water, the preparation dried between blotters, mounted, and examined by an oil-immersion. For mounting use xylol-dammar, as the ordinary Canada balsam bleaches these preparations. Usually any precipitate which forms can be removed with the stream of water. If especially clean and clear specimens are desired, the precipitate may

[9] Medical News, 1903, lxxxiii, 248.

be removed by momentary immersion in 95 per cent. alcohol, which should be immediately washed off with distilled water. By this method the spirochætes appear a light carmine color.

Method of R. Kolb.[10]—Kolb describes a staining method which, he states, takes less than one minute. The technic is recommended especially to the general practitioner for its simplicity and convenience. The stain is a combination of 0.5 part eosin B. A., 50 parts alcohol, and 40 parts Ehrlich's triacid stain (see Appendix for preparation). The stain thus contains four dyes; the mixture should be clear. He prefers oozing serum for the test, obtained after a papule is rubbed a little or an erosion curetted. The specimen is fixed, a few drops of the stain are poured on it, and the whole heated over a flame; then rinsed with water, after which a large amount of a 10 per cent. solution of commercial (8 per cent.) acetic acid is poured over it carefully two or three times from the edge. The specimen should be uniformly pink, while the spirochætes and bacteria do not take the stain, but show up white against the pink background, unless the staining is too intense, in which case it is better to use a new smear and shorten the staining time. The spirochætes seem to show up better in the edges of the preparation.

India Ink Method.—Burri advanced this method, which is simple and reliable, although some specimens of ink may show confusing artefacts, due, according to Barach, to the use of an inferior ink. A drop of the fresh serum from the lesion is placed at one end of a glass slide and immediately mixed with a small drop of Gunther-Wagner India ink (chin-chin liquid pearl ink). The mixture is then spread and allowed to dry. The specimen is studied with the immersion lens. The whole field is a homogeneous brown or black color. The treponema, blood-cells, etc., appear as colorless, highly refractile bodies on a homogeneous black or brown field.

METHOD FOR RAPID STAINING OF LIVING SPIROCHAETES.

Meirowsky[11] mixes methyl-violet stain with a few drops of physiologic salt solution and rubs the colored mixture vigorously

[10] Münch. med. Wochen., June 28, lvii, No. 26.
[11] *Ibid.*, July 5, lvii, No. 27.

into the ulcerated primary sore or condyloma. As serum oozes it is found to contain the spirochætes stained a bright violet. The depth of the stain depends on the concentration of the coloring mixture; it works best when it is of such a strength that the liquid envelope of the red corpuscles is a deep bluish-violet tint. The refringens assume a bluish-violet tint, especially distinguished from the bright violet stain of the pallida.

By the above methods of examination it will usually be found that the organisms are most numerous in moist papules and chancres (when the curettage is carried out at the edge of the lesion). In scrapings from roseola the search is frequently disappointing. The organism should be distinguished from *S. refringens, S. dentium, S. mucosum,* etc.

This section would not be complete without mention of the recent and important researches of Noguchi, who has succeeded in culturing the organism of syphilis.[12] The following is a synopsis of his findings:—

"The standards by which I identify *Spirochæta pallida* in cultures are: (1) correct morphology; (2) necessity of the presence of sterile fresh tissue in culture medium; (3) strict anaërobiosis; (4) rather faint hazy growth in solid or fluid mediums, without any noticeable change in the proteid constituents; (5) non-production of any offensive odor in culture; (6) capability of inciting an allergic reaction on the skin of certain cases of syphilis and parasyphilis (so-called luetin reaction); (7) specific complement fixation with the antipallida immune serum of certain serums from human cases of syphilis, provided that the antigen is suspended in saline solution and not prepared by an alcoholic extraction, and (8) pathogenicity. The pathogenicity may be gradually attenuated in course of cultivation, but the other seven conditions should be constantly fulfilled.

"There are some small spirochæte varieties which are difficult to differentiate from the pallida. Morphologically, *Spirochæta microdentium* and *Spirochæta mucosa* closely resemble the pallida, but both of the former can grow in ascitic agar without the addition of fresh tissue and produce an offensive odor. Neither variety binds complement with the antipallida immune

[12] Jour. Amer. Med. Assoc., Oct. 5, 1912, vol. lix, No. 14.

serum when used in an aqueous suspension. From the appearance of the growth *Spirochæta microdentium* and *Spirochæta refringens* closely resemble the pallida, but they are quite different in morphologic features. Neither of these organisms produces any odor in culture and the *S. refringens* grows without the fresh tissue."

Examination by Dark-field Illumination (see Section I, page 7).

ANIMAL PARASITES.

Temporary parasites include the ectoparasites (epizoa). These may inhabit the skin, conjunctival sac, the mouth, the nose, and the accessory sinuses. A familiar example of this class is the *Sarcoptes scabiei,* or itch mite. (See page 159, etc.)

Most of the **permanent** or **stationary** parasites are found in the internal organs, and belong to the class of entoparasites or entozoa. Many of these parasites, such as the teniæ, ascarides, and ankylostoma, inhabit man only when mature; others, of which the echinococcus is an example, inhabit man only during a certain period of their existence. Thus, man may be either the actual or only the intermediate host. Again, for many parasites, such as the *Tenia solium* and the *Tenia saginata,* man is the only host. Finally, man may also become the host for parasites which, as a rule, select some other animal. Thus, the *Cysticercus cellulosæ,* common to the pig and the cat, may occasionally be found in man.

In this section the classification of Max Braun[13] has been adopted, and the majority of the descriptions of the parasites which follow have been abstracted from that work.

No attempt has been made to include the rarer forms of parasites, as these are considered to be beyond the scope of this work.

CLASSIFICATION OF THE MORE COMMON ANIMAL PARASITES OF MAN.

A. Protozoa.

CLASS I. RHIZOPODIA.—Ameba coli (*Loesch*).

[13] English translation of "The Animal Parasites of Man." (Wood & Co., 1906.)

Class II. Flagellata (Mastigophora).

(a) *Trichomonides:*

1. Trichomonas vaginalis.
2. Trichomonas intestinalis.
3. Trichomonas pulmonalis.

(b) *Circomonides:*

1. Lamblia intestinalis.
2. Trypanosoma.

Class III. Sporozoa.

Coccidia:

1. Coccidium perforans or hominis.
2. Hemosporidia.

Class IV. Infusoria.—Balantidium coli or paramecium coli.

B. Platyhelminthes (*Flat worms*).

Class I. Trematoda (*Rud*).

1. Fasciola hepaticum *syn.* distomum hepaticum.
2. Distomum pulmonale *syn.* distomum Westermani.
3. Distomum lanceolatum *syn.* dicrocelium lanceolatum.
4. Distomum hematobium *syn.* bilharzia, *syn.* schistosomum hematobium.

Class II. (*Rud.*)

(a) *Bothriocephaloidia:*

Bothriocephalus latus *syn.* tenia lata.

(b) *Teniidæ:*

1. Tenia nana.
2. Tenia lanceolata.
3. Tenia solium.
4. Cysticercus acanthotrias.
5. Tenia saginata or mediocanellata.
6. Tenia echinococcus.

C. Nematoda (*Thread worms*).

1. Strongyloides (rhabdonema strongyloides) *syn.* anguillula intestinalis et stercoralis.

2. Filaria sanguinis hominis.
3. Trichocephalus dispar (*whip worm*).
4. Trichina spiralis.
5. Ankylostoma duodenale.
6. Uncinaria Americana.
7. Ascaris lumbricoides.
8. Oxyuris vermicularis.

A. PROTOZOA.

The protozoön is a microscopic living organism. It is monocellular and represents the lowest form of animal life. The substance of the body consists of a finely granular, contractile protoplasm, which may be mono- or poly- nuclear. The viscid hyaline entosarc is capable of motion by expansion and contraction, or by the extension and retraction of pseudopodia, cilia or flagella. Propagation takes place by segmentation or gemmation.

CLASS I. RHIZOPODIA.

The ameba coli produces the well-known amebic dysentery. The ameba in man is, however, not confined to the intestines, but has been found in the pus of liver abscesses, in pleuritic and peritoneal exudates, in the mucous membranes, and in tumors of the urinary bladder.

Characteristics.—This organism is an ameboid body measuring from 20 to 30 microns in diameter. It is composed structurally of a clear protoplasmic outer portion, ectosarc, and a finely or coarsely granular central portion, entosarc, which usually shows a number of clear vacuoles and one or more nuclei (Plate V, *a*). When living it shows active ameboid movements which are greatly increased if the organism is kept warm. In the living state the cell frequently includes foreign bodies, such as bacteria, pigment granules, and fragments of blood-corpuscles and other cells.

Locomotion is accomplished by irregular extension and retraction of pseudopods, which are thrown out from the periphery. These pseudopods are at first composed of the clear outer portion, which, as the projection gradually increases, includes the central granular zone. When surrounded by unfavorable envi-

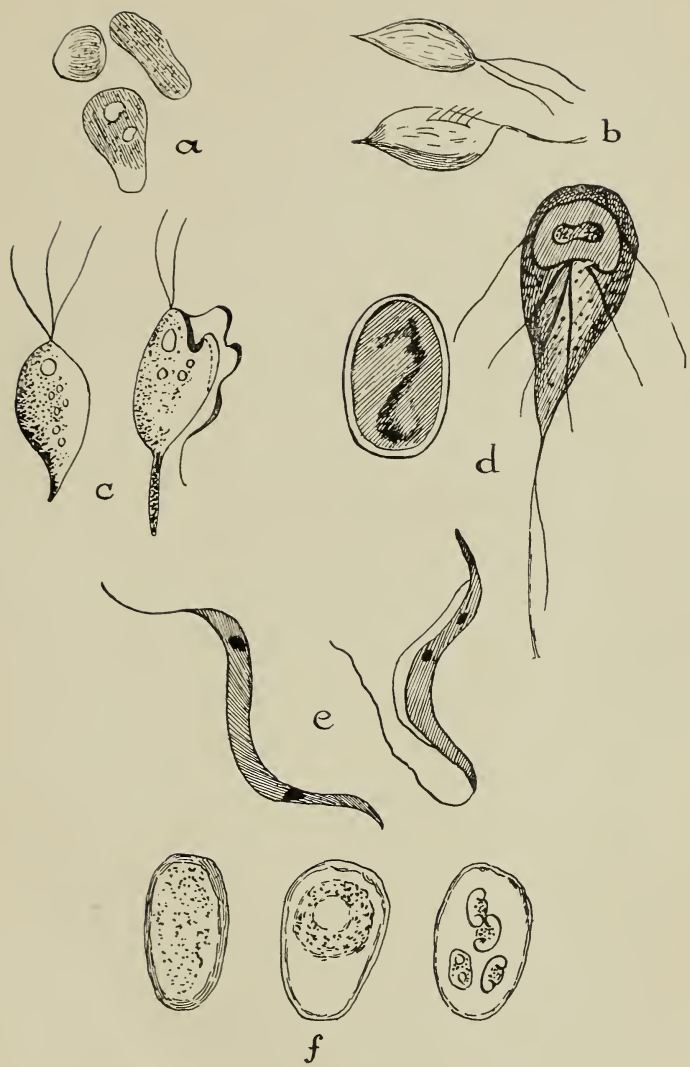
ronment, the organism undergoes a form of change known as the *encysted state.* In this state the body becomes spherical and the outer wall thickened, while the division of the cell into two portions is lost, the whole becoming uniformly granular.

Method of Examination.—The fecal discharges should be caught in a warm receptacle and immediately transferred to the laboratory for examination. If it is necessary to keep the specimen for a short time, this may be accomplished by immediately placing the specimen in a thermostat at body temperature, where it should remain until transferred to the warm stage of the microscope. Preservation of specimens for more than a few hours is unsatisfactory, because even under the favorable circumstances of heat and moisture obtaining in the thermostat, motility is rapidly lost, and at the expiration of twenty-four or thirty-six hours the organisms are no longer discoverable.

The Warm Stage.—A convenient method of maintaining a warm stage for examination and study of this organism is as follows: A flat strip of copper or of brass, three inches wide by six or eight inches long, is perforated by a half- or three-quarter inch aperture, situated in the center of one end at a distance of about one inch from the free margin. The stage of the microscope should be covered with several thicknesses of asbestos paper or felt, upon which the metal sheet is clamped so that the openings in the stage and in the copper-strip coincide. A Bunsen burner or alcohol lamp is adjusted under the outer extremity of the strip, so that when the metal has attained its maximum heat the portion immediately surrounding the aperture is maintained at about body-temperature. A specimen placed upon a slide, in position for examination, will maintain the motility of the ameba for a number of hours.

It will be found convenient to examine the spread, first by the low power, then, after locating a suitable portion of the slide, the higher objective may be swung in and the organisms studied in detail. The important aids to successful search are a thin and uniform spread of liquid feces, and a low, slightly oblique illumination. The microscope should be as near the patient as possible. A high rectal tube is passed, and the fecal matter, often containing blood or mucus, found in the eyelets of the tube is transferred to a warm slide, covered with a warm

PLATE V



INTESTINAL PARASITES OF MAN. (*Redrawn from Max Braun*)

a, Amebae coli. *b*, Trichomonas vaginalis. *c*, Trichomonas intestinalis *d*, Lamblia intestinalis. *e*, Tripanosomes. *f*, Coccidium hominis.

cover-slip, and immediately studied. In case it is necessary to carry the tube to a laboratory, this is best done by arranging two pans like a chafing-dish, the lower one containing water at about 100° F., the upper one resting on the water, and containing the tube. Before the diagnosis of amebæ is justified, one must find unicellular organisms, showing actively motile pseudopodia, and moving from place to place by the activity of the pseudopodia.

CLASS II. FLAGELLATA.

a. Trichomonides.

1. Trichomonas Vaginalis (see Plate V, *b*).—The form of the body is very variable, elongated, fusiform or bean-shaped. It is ameboid. The length varies between 0.015 and 0.025 millimeter in length, by 0.007 to 0.012 in breadth. The posterior extremity is drawn out into a point and is about one-half the length of the remainder of the body. The cuticle is very thin and the body-substance finely granular. At the anterior extremity there are three or four flagella, which are of equal length, and which are firmly united at their base, but which easily fall off. There is an undulating membrane which moves spirally across the body, arising from the base of insertion of the flagella and terminating at the base of the caudal process. The nucleus is vesicular, elongated, and situated in the anterior extremity. Propagation occurs by division.

2. Trichomonas Intestinalis (see Plate V, *c*).—Some authors believe this organism to be identical with the *Trichomonas vaginalis*. It is described as being 0.01 to 0.015 millimeter in length; the posterior extremity terminates in a point with a row of cilia. This organism has been found in the urethra, the vagina, the large and small intestines, the stomach, and in the oral cavity.

3. Trichomonas Pulmonale. In all probability this is identical with the preceding.

b. Cercomonides.

1. Cercomonas Intestinalis or Lamblia Intestinalis (see Plate V, *d*).—Length, 0.01 to 0.02 millimeter, and width, 0.005 to 0.012. The flagella are of about equal length (0.009 to 0.014 millimeter). The body is finely granular, and has a very thin cuticle, which does not entirely prevent changes in the form of the body. The very motile, tail-like appendages in the frontal

plane are flattened; the excavation at the anterior extremity which is directed obliquely forward and with its border projecting backward. The anterior pair of flagella arise from the anterior edge of the peristome; the lateral and median from the posterior edge, whereas the tail flagella are inserted at the posterior end. The anterior flagella appear to be connected with the nucleus. The nucleus is dumb-bell shaped and has a nucleolus in each half, and lies anteriorly in the part of the body which is excavated. This organism has *encysted stages*. These *cysts* are oval and measure .01 by .007 millimeter. They are surrounded by a fairly thick hyaline layer through which the outline of the creatures can sometimes be seen quite distinctly.

Trypanosoma.—The Trypanosome[14] has a more or less spindle-shaped body, along one border of which runs an undulating membrane. There is a nucleus and a blepharoblast, the latter being located anteriorly as a chromatin staining dot or rod. From this blepharoblast the flagellum proceeds posteriorly bordering the undulating membrane and projecting freely beyond the posterior end. The nucleus is larger, nearer the posterior end, and does not stain so intensely as the blepharoblast.

T. Gambiense.—This is the trypanosome causing human trypanosomiasis, the later stage of which is known as sleeping sickness. It is from 17 to 28 μ long, and from 1.5 to 2 μ wide.

It is very difficult to distinguish the human trypanosome from some of the other pathogenic ones by staining methods.

It is present in the blood, usually in exceedingly small numbers, and in the lymphatic glands of patients. It is by puncture of the glands that we have the best means of finding the parasites. The parasite stains readily with Wright's stain. The transmitting agent is the Glossina palpalis. It is not known whether this occurs by direct or indirect transmission. At any rate, no tsetse, no trypanosomiasis.

CLASS III. SPOROZOA.

Coccidia.

1. Coccidium Hominis or Perforans.—This organism is oval. The fertilized sporont stage is the oöcyte (see Plate V, *f*), and measures .024 to .035 by .002 to .014 millimeter. It is surrounded by an integument with a double outline which has an

[14] E. R. Stitt: "Practical Bacteriology," etc., 1909.

opening at the pointed pole. The plasm, which is somewhat coarsely granular, entirely fills the integument or is gathered together in a rounded central mass. The coccidia are evacuated from the bowel in this stage, and sporulate in the open within two or three weeks. The fully developed *spores* are of a broad fusiform shape and measure .012 to .015 millimeter in length by .007 millimeter broad. They each contain two *sporozoites,* broad at one end and pointed at the other, forming a bent dumb-bell shaped body. The chromatin mass is called the *centrosome,* and the extremity of the body which encloses this, is the anterior extremity.

A granular residual body lies in the concavity. This organism is found in the intestinal tract, where it may give rise to violent auto-infection and chronic diarrhea.

2. Hemosporidiæ.—These organisms are the cause of malaria and have been described in another section (see page 126).

CLASS IV. INFUSORIA.

Balantidium Coli or Paramecium Coli.—The *body* is oval, .06 to 1.0 millimeter in length, by .05 to .07 millimeter in width. The peristome is funnel-shaped or contracted, the anterior end being either blunt or pointed. The ecto- and entosarc are distinctly separate, the latter granular, and containing drops of fat, mucus, starch granules, bacteria, and occasionally leukocytes and erythrocytes. There are usually two contractile vacuoles. The anus opens at the posterior extremity, and the organism contains a macro- and a micro-nucleus. It occurs in the large intestines of man.

B. PLATYHELMINTHES (Flat Worms).

CLASS I. TREMATODES.

1. Distomum Hepaticum *(Liver fluke).*—Length, 20 to 30 millimeters; breadth 8 to 13 millimeters. The *head-cone* is 4 to 5 millimeters long, and sharply demarcated from the posterior part of the body. *Spines* in alternating transverse rows, and extending on the ventral surface to the posterior border of the testes, and on the dorsal surface not quite so far. The spines are smaller on the head-cone than on the posterior part

of the body, where they are discernible by the naked eye. The *suckers* are hemispherical and near each other. The oral sucker is about 1 millimeter, and the ventral about 1.6 millimeter in diameter. The *pharynx,* which includes almost the entire esophagus, measures .7 millimeter in length and .4 millimeter in breadth. The intestine bifurcates in the head-cone, and the branches are furnished with blunt sacs directed outwardly. The *ovary* is ramified and is situated in front of the transverse vitello duct. The shell-glands lie near the ovary and are in the median line.

The *ova* are yellowish-brown, oval, with cap-like lid. They measure .130 to .145 by .07 to .09 millimeter. The average size is .132 by .08. The liver fluke is an inhabitant of the bile-ducts of man.

2. Distomum Pulmonale or Distomum Westermanni.—The *body* is of a faint, reddish-brown color and plump oval shape, with the ventral surface a little flattened. This organism measures 8 to 10 millimeters in length by 4 to 6 millimeters in thickness. It possesses *two suckers* of equal size (.75 millimeter).

The *eggs* are oval brownish-yellow, with a fairly thin shell and measure .0875 to .1025 millimeter in length, by .052 to .075 millimeter in breadth, the average being .0935 by .057 millimeter. Their location is usually in the lung, but they may enter the blood-vessels and be carried to another part of the body.

3. Distomum Lanceolatum or Dicrocelium Lanceolatum.—In the fresh condition this is a yellowish-red organism, the *body* is flat, almost translucent, with a conical neck at the level of the ventral sucker. This point is marked by a shallow constriction. The length and breadth vary according to the amount of contraction, being usually 8 to 11 millimeters by 1.5 to 2.0 millimeters. The *suckers* are about one-fifth of the body-length distant from each other, and of about equal size (.23 by .25 millimeter).

The *eggs* are oval with a sharply defined operculum at the pointed pole. They measure .030 by .011 millimeter, and occur in the feces. The parasites reside in the liver.

4. Distomum Hematobium or Bilharzia.—The *male* is

whitish and measures 12 to 14 millimeters in length, but is already mature when 4 millimeters long. The anterior end is 6 millimeters or a little more in length; the suckers are situated near each other; the oral sucker is infundibuliform; the dorsal lip being a little longer than the ventral. The ventral sucker is slightly the larger, and is pedunculated. A little behind the ventral sucker the body broadens to a width of 1 millimeter, decreasing, however, in thickness. The lateral edges curl ventrally, so that the posterior part of the body is almost cylindrical. The posterior end is somewhat attenuated, and the dorsal part of the posterior extremity of the body is covered with spinous papilli.

The *females* are filiform, about 30 millimeters in length, pointed at each end and measuring .25 millimeter in diameter. The color of the body varies according to the contents of the intestine (posteriorly they are dark-brown or black). The cuticle is smooth except in the suckers, where there are very delicate spines, and at the tail end where there are larger spines. The anterior part of the body measures from .2 to .3 millimeter in length.

The *eggs* are fusiform and much dilated in the middle. They have no lid and are provided with a terminal spine at the posterior extremity. The eggs *measure* 0.12 to 1.12 millimeters in length, by .05 to .073 millimeter in breadth. They are yellowish in color, slightly transparent, and provided with a thin shell. The spine may sometimes be absent. The eggs apparently vary greatly in size. The organism lives in the portal vein and its branches.

CLASS II.

a. Bothriocephaloidia.

1. Bothriocephalis Latus or Tenia Lata. — *Length* from 2 to 9 or more millimeters. Color, yellowish gray; after lying in water lateral areas become brownish and the rosette of the uterus brown. The *head* is almond-shaped, 2 to 3 millimeters in length. Its dorso-ventral axis is longer than its transverse diameter; the head is therefore generally flat, concealing the suctorial grooves at the borders. The neck varies in length according to the degree of contraction, and is very thin. There

are from 3000 to 4500 *proglottides.* Their breadth is usually greater than their length, but in the posterior third of the body they are almost square, while among the very oldest some may be longer than they are broad.

The *eggs* are large with brownish shells and small lids. They measure .068 to .071 by .045 millimeter. The proglottides near the posterior extremity of the worm are frequently eggless.

b. Teniidae.

1. Tenia Nana.—The worm is 10 to 15 millimeters in length, and .5 to .7 millimeter in breadth. The *head* is globular and is from .25 to .30 millimeter in diameters. The rostellum has a simple crown consisting of 24 to 30 hooks, which are only .014 to .018 millimeter in length. The neck is moderately long. The *proglottides* are very narrow, about 150 in number, .4 to .9 millimeter in breadth, by .014 to .030 millimeter in length.

The *eggs* are globular or oval, and measure .030 to .048 millimeter. The oncospheres measure .016 to .019 millimeter in diameter.

The worm lives in the intestines; the ova, and proglottides are found in the feces.

2. Tenia Lanceolata or Hymenolepis Lanceolata.—The parasite measures 30 to 130 millimeters in length, and 5 to 18 millimeters in breadth. The *head* is globular and very small, the rostellum is cylindrical with a crown composed of eight hooks (0.031 to 0.035 millimeter in length). The neck is very short. The segments increase gradually in breadth, but vary little in length.

The *ova* have three envelopes and are oval, measuring 0.050 by 0.035 millimeter. The external envelope is membranous and much wrinkled, the middle one is thick, and the internal one very thin.

3. Tenia Solium or Tenia Vulgaris. — The average length of the entire tapeworm is about 2 to 3 meters, but may be more. The *head* is globular, 0.6 to 1.0 millimeter in diameter. The rostellum is provided with a double row of hooks, twenty-two to thirty-two in number; large and small hooks alternate regularly. The length of the largest hooks is 0.16 to

0.18 millimeter, of the small ones 0.11 to 0.14 millimeter. The average number of *proglottides* is 800 to 900; they increase very gradually in size. At about 1 millimeter behind the head they are square and have the generative organs fully developed. Segments sufficiently mature for detachment measure 10 to 12 millimeters in length, by 5 to 6 millimeters in breadth. The fully devloped uterus consists of a median trunk with seven to ten lateral branches on each side, some of which are again ramified.

The *eggs* are oval, the egg-shell very thin and delicate. The *embryonal shell* is very thick with radial stripes; it is of a pale-yellow color, globular, and measures 0.031 to 0.036 millimeter in diameter. The *oncospheres* with six hooks are likewise globular, and measure 0.02 millimeter in diameter.

4. Cysticercus Acanthotrias.—This resembles the *cysticercus cellulose* in form and size, but carries on the rostellum a triple crown each consisting of fourteen to sixteen hooks which differ from the hooks of the cysticercus cellulose or of the tenia solium by the greater length of the posterior root process and the more slender form of the hooks. The large hooks measure 0.153 to 0.196 millimeter, the medium-size hooks 0.114 to 0.14, and the small ones 0.063 to 0.07.

5. Tenia Saginata or Tenia Mediocanellata. — The length of the entire worm averages 4 to 8 to 10 meters and more, even up to 36 meters. The *head* is cuboid in shape, 1.5 to 2 millimeters in diameter. The suckers are hemispherical (0.8 millimeter), and are frequently pigmented. There is a sucker-like organ in place of the rostellum, and this is also frequently pigmented. The neck is moderately long and about half the breadth of the head. The *proglottides* average more than 1000, and gradually increase in size from the head backward. The detached mature segments are exactly like pumpkin-seeds—they are about 16 to 20 millimeters long, by 4 to 7 millimeters broad. There are twenty to thirty-five lateral branches at each side of the uterus, and these often again ramify.

The *eggs* are more or less globular, the egg-shell frequently remains intact, and carries one or two filaments. The *embryonal shell* is thick, radially striated, transparent and oval. It is 0.3 to 0.4 millimeter in length, by 0.02 to 0.03 millimeter in breadth.

This worm in the adult condition dwells exclusively in the intestinal canal of man. The corresponding *cysticercus* occurs in the ox and steer.

6. Tenia Echinococcus.—This worm measures 2.5 to 5 or 6 millimeters in length; the *head* is 0.3 millimeter in breadth, and has a double row of twenty-eight to fifty hooklets on the rostellum. The size and form of these hooklets vary. The larger ones are 0.040 to 0.045 millimeter in length, the smaller ones are 0.030 to 0.038 millimeter. The suckers measure 0.13 millimeter in diameter. The neck is short, behind which there are only three or four segments, the posterior of which is about 2 millimeters in length and 0.6 millimeter in breadth. The ovary is horse-shoe shaped, with the concavity directed backward. The median trunk of the uterus is dilated when filled with eggs, and instead of lateral branches has lateral protuberances. It is not uncommon for the *eggs* to form local heaps. The *embryonal shell* is moderately thin with radiating fibers, is almost globular, and measures 0.030 to 0.036 millimeter in diameter.

The mature parasite lives in the small intestine of the domestic dog and the wolf, and from them, the dog chiefly, they are transmitted to man.

Diagnosis of Cestodes.—The microscopic examination of the feces should never be neglected when the presence of tapeworm is suspected. Often by careful, frequently repeated examinations, insistent symptoms, referable to the digestive tract, the nervous system or the general nutrition, may be cleared up by the finding of segments or ova in the feces.

As the uterus of the tenia has no exit, the eggs can only find egress when the mature proglottide is injured. In the case of the tenia saginata the discharge of eggs is almost the rule. The proglottides when discharged are usually without eggs. The eggs of the solium and the saginata are only distinguished by their size.

In examining the stools for evidences of tapeworms, one must be careful not to confound remnants of undigested food, mucous casts, and shreds of tendon with the proglottides. The proglottides, after being soaked in water, assume their characteristic form. As a rule the microscopic determination of ova is a more certain means of diagnosis than the macroscopic segments.

To determine from the shape of the proglottide which variety of worm is present, it is advisable to fix the segment between two glass slides. The proglottide of the tenia solium is more delicate and more transparent than the tougher segments of the tenia saginata. In the former the branching uterus is more plump, and the number of lateral twigs are from seven to ten, while the uterus of the tenia saginata shows from twenty to thirty or more.

C. NEMATODES (Thread Worms).

Strongyloides Intestinalis or Anguillula Intestinalis et Stercoralis.—1. (a) The *parasitical generation (anguillula intestinalis)* measures 2.3 millimeters in length by 0.034 millimeter in breadth. The cuticle is finely transversely striated. The mouth is surrounded by four lips, the esophagus is almost cylindrical and is a quarter the length of the body. The anus opens just in front of the pointed posterior extremity.

The *eggs* measure 0.050 to 0.58 millimeter in length, and 0.030 to 0.034 in breadth.

(b) The *free-living generation (anguillula stercoralis)* is sexually differentiated. The body of the *male* is cylindrical, smooth, somewhat more slender at the anterior extremity, and pointed at the tail end. The mouth has four lips and the esophagus a double dilatation. The males measure 0.7 by 0.035 millimeter, and carry the posterior extremity curled up. The two spicules are small and much curved. The *females* measure 1.0 millimeter in length or a little more, 0.05 millimeter in breadth. The tail end is straight and pointed. The yellowish thin-shelled *ova* measure 0.07 millimeter in length by 0.045 millimeter in breadth.

2. Filaria Sanguinis Hominis *(filaria bancrofti or filaria nocturna).*—The *male* is colorless and measures 40 millimeters in length and 0.1 millimeter in diameter. The *cephalic extremity* is a little thickened, the posterior extremity is bent and rounded, but is not twisted cork-screw like. The anal orifice opens 0.138 millimeter in front of the posterior border. The *female* is brownish, 7.8 to 8.0 millimeters in length and 0.21 to 0.28 millimeter in breadth. The cephalic and caudal extremi-

ties are rounded. Almost the entire body is occupied by the two uteri, from which the larvæ emerge early. The length of the *larvæ* average 0.13 to 0.3 millimeter, their breadth 0.007 to 0.07 millimeter. They are surrounded by a delicate protective investing membrane which is not quite close to them.

The lymphatic vessels in various parts of the body are doubtless the normal habitat of the adult worms, but these have also been found in the left ventrice of the heart. The young ova, by means of the lymph-stream, reach the blood and are distributed with it through the body. They also pass through the vessel walls and may be found in the fluid of the glands of the body. The larvæ are first found in infected patients only in specimens of blood that have been taken after sunset. Their number increases considerably until after midnight, and after that time begin to diminish. From mid-day until evening no filariæ are found in the peripheral blood.

3. Trichocephalus Dispar or Ascaris Trichiura.—The *male* measures 40 to 45 millimeters in length, the spiculum is 2.5 millimeters long and lies within a retractile pouch beset with spines. The *female* measures 45 to 50 millimeters in length, of which two-fifths appertain to the posterior part of the body. The ova are barrel-shaped and have a thick, brown shell which is perforated at the poles. Each opening is closed with a light-colored plug. The *eggs* measure 0.05 to 0.054 millimeter in length and 0.023 millimeter in breadth. They are deposited before segmentation. This worm usually lives in the cecum of human beings, and is occasionally found in the vermiform appendix, in the colon, and exceptionally in the small intestine. Usually only a few are present, and they do not cause any particular disturbance.

The *development of the eggs* is completed in water or in moist soil, and occupies a longer or shorter period according to the season. The eggs and larvæ possess great powers of resistance, and have been known to remain as long as five years in the egg-shell without losing their vitality.

4. Trichina Spiralis.—The *male* measures 1.4 to 1.6 millimeters in length and 0.04 millimeter in diameter. The anterior part of the body is narrowed, the orifice of the cloaca is terminal and lies between the two caudal appendages; behind

there are four papillæ. The *female* measures 3 to 4 millimeters in length and 0.06 millimeter in diameter; the anus is terminal. Trichina spiralis occupies in its adult stage the small intestines of men and of various mammals, including the domestic rat, domestic pig and domestic dog.

History of Development of Trichina Spiralis.—Shortly after entering the intestines the encysted trichinæ escape from their capsules, and then enter the duodenum and jejenum where they become adult. During this period they do not greatly increase in size. The males grow from 0.8 to 1.0 millimeter, the females from 1.5 to 1.8 millimeters. Soon after copulation, which takes place in the course of two days, the males die off, and the females, which soon attain the length of 3.0 to 3.5 millimeters, either bore more or less deeply into the villi or penetrate the mucous membrane and enter the lymphatic spaces. Here they deposit their young which, according to Leuckart, average at least 1500. The migrations are mostly passive, the larvæ being carried along by the lymph-stream or by the circulating blood. The young brood is distributed throughout the entire body, but the conditions necessary to its further development, are found only in the transversely striated muscle. On the ninth or tenth day after infection the first trichinæ have reached their destination, but further invasions are constantly taking place. Two or three weeks after infection the spirally rolled up trichinæ have grown to 0.8 to 1.0 millimeter, and in their vicinity the muscle fibers are swollen. The capsule is formed by the inflamed connective tissue producing the cystic membrane. The *cysts* are lemon-shaped and usually lie with their longitudinal axis in the direction of the muscle fibers. On an average they measure 0.4 millimeter in length by 0.25 millimeter in breadth.

5. Ankylostoma Duodenale or Uncinaria Duodenale.—The body is cylindrical, attenuated anteriorly, and of a slightly reddish color. In the oral cavity on the ventral surface, close behind the orifice, are four hook-like teeth directed backward; on the dorsal surface there are two teeth directed forward. The *males* measure 8 to 10 millimeters in length, and 0.4 to 0.5 millimeter in breadth. The bursa has two large lateral and one small dorsal alar processes. The *females* measure 12 to

18 millimeters in length, and the caudal extremity has a small spine. The *eggs* are elliptical and have thin shells; they measure 0.032 to 0.045 millimeter in breadth, and 0.055 to 0.065 millimeter in length.

The ankylostoma duodenale lives in the duodenum, and may rarely be found in the first part of the jejunum.

6. Uncinaria Americana can readily be distinguished from the preceding worm. It is shorter and more slender. The *male* worm measures from 7 to 9 millimeters in length by 0.3 to 0.35 in diameter; the *female* 9 to 11 millimeters in length by 0.4 to 0.45 millimeter in diameter. The buccal capsule is much smaller, and presents an irregular border; instead of four ventral hook-like teeth, it is provided with a vertical pair of prominent semilunar plates similar to those of a dog hook-worm. The pair of dorsal teeth is likewise represented by a pair of slightly developed chitinous plates of the same nature.

The *eggs* are larger than in U. Duodenale; they measure 64 to 75 micromillimeters by 36 by 40 micromillimeters in breadth. So far this worm has been found only in man; its anatomical *habitat* is the small intestine.

7. Ascaris Lumbricoides.—The coloring in the fresh stage is reddish- or grayish-yellow. The body is of an elongated spindle shape and the dorsal oral papillæ carries two papillæ of sense and the two ventral oral papillæ are papillæ of sense. The *male* measures from 15 to 25 centimeters in length and about 2 millimeters in breadth. The posterior extremity is conical and bent ventrically into a hook. The *spicules* measure 2 millimeters in length and are curved and broadened at their free ends. On each side of the orifice of the cloaca are seventy to seventy-five papillæ, of which seven pairs are post-anal.

The *female* measures 20 to 40 centimeters in length and about 5 millimeters in diameter, the posterior extremity is conical and straight. The vulva is at the border between the middle and posterior thirds of the body, from which the two uterine tubes pass straight to the posterior end of the body. The convoluted ovaries measure ten times the length of the body.

The *ova* are elliptical with a thick transparent shell and an external coating of albumin which forms protuberances. The ova measure 0.05 to 0.07 millimeters in length and 0.04 to 0.05

millimeter in breadth; they are deposited before segmentation. The albuminous coating is stained yellow by the coloring matter of the feces.

This worm is one of the most frequent parasites of man, and is distributed over all parts of the world.

8. Oxyuris Vermicularis or Ascaris Vermicularis.—Color, white; the attenuated cuticle forms swellings at the anterior end which extend some distance back along the middle of the ventral and dorsal surfaces. There are three small retractile labial papillæ around the mouth. The *male* measures 3 to 5 millimeters in length, and shortens on death. The posterior extremity of the body is rolled ventrally and presents papillæ. The *female* is 10 millimeters in length and 0.6 millimeter in diameter. The anus is about 2 millimeters in front of the tip of the tail; the vulva is in the posterior third of the body. The *eggs* are oval, thin-shelled, and measure 0.05 by 0.02 millimeter. They are deposited with embryos already developed, and are seldom found in the feces.

TEMPORARY PARASITES.

PARASITES OF THE SKIN.

Arthropoda.

These are bilaterally symmetrical, segmented animals whose segments do not correspond. The segments are often more or less fused, thus forming special body-regions which may themselves be more or less fused together as well. The arthropods commonly reproduce by ovulation, the development of the embryo to the adult often showing more or less complicated metamorphoses. The true parasitic forms of the arthropoda thus far met in man are limited to the arachnoids and insects.

Arachnoidea.

Sarcoptes or Acarus Scabiei (the Itch Parasite).—This parasite is oval in shape, is provided with horns and bristles (see Fig. 30), and is barely visible to the naked eye. The male measures from 0.2 to 0.3 millimeter in length by 0.145 to 0.19 millimeter in breadth; the female is somewhat larger, showing

a length of 0.33 to 0.45 millimeter and a breadth of 0.25 to 0.35 millimeter.

The female lies at the end of a burrow in the epidermis, in situations where the skin is most delicate, as between the fingers, at the elbows, under the knees, and in the groin. In this burrow, which varies from a few millimeters to a centimeter in length, the female deposits her eggs, after which she dies. The eggs hatch in from four to eight days, and in about fourteen days the larvæ are sufficiently matured to make their own burrows. The disease is communicated either by the clothing or by personal contact. To demonstrate the parasite, the burrow is

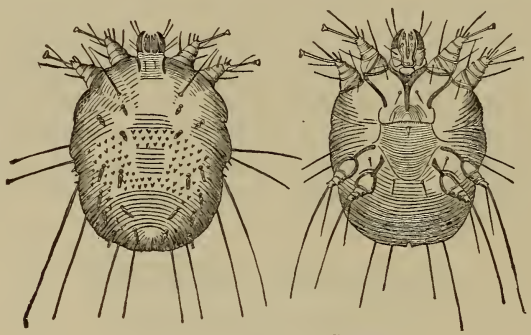


FIG. 30.—SARCOPTES SCABIEI.

opened with a needle and the female is pressed out on a slide, which is then covered and examined.

Demodex Folliculorum.—This parasite is very small, varying in length from 0.3 to 0.4 millimeter. It is somewhat cylindrical, tapering to an obtuse point at the posterior end. This parasite has its habitat in the sebaceous follicles, especially of the face and nose.

Leptus Autumnalis (Harvest Bug).—This is a minute red parasite, from 0.3 to 0.5 millimeter long, which has three pairs of legs, with a row of bristles upon its back and belly. It prevails in summer on grass and plants and attaches itself to the skin of man by its hooklets.

Insecta. Hemiptera.

Pediculus Capitis (Head Louse).—The male is from 1 to 1.5 millimeters long; the female is 1.8 to 2 millimeters long. The

color of the parasite varies somewhat with the race of its host. In the Caucasian it is gray with a dark border; in the Negro and Chinaman it is much darker in color. The eggs are 0.6 millimeter in length and are attached to the hairs, forming the so-called "nits." These nits are whitish, oval masses which are easily visible. This parasite, while usually found upon the hair of the head, may be found in other portions of the body.

Pediculus Vestimenti (Body Louse).—This parasite is considerably larger than the former, being from 2 to 5 millimeters long and whitish gray in color, the back part of the body being wider than the thorax. The antennæ are longer than those of the head louse. The eggs are from 0.7 to 0.9 millimeter in

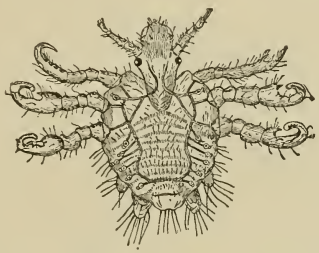


FIG. 31.—PEDICULOS PUBIS.

length, about 70 being laid by each female. This parasite is found upon the clothing, in which it deposits its eggs, especially about the neck, back, and abdomen.

Pediculus Pubis (Phthirius Inguinalis, or Crab Louse).—This parasite is smaller than the head louse (see Fig. 31), grayish yellow or gray white in color, the male being from 0.8 to 1 millimeter in length, the female about 1.12 millimeters in length. The eggs are pear-shaped, from 0.8 to 0.9 millimeter in length and from 0.4 to 0.5 millimeter in breadth.

This parasite infects the parts of the body covered by the shorter hairs, such as the pubis, axilla, eyebrows, and chest.

Cimex Lectularius (Acanthia Lectularia, or Bedbug).—While, strictly speaking, the bedbug is not a parasite of man, yet as its habitat is the bed, bedding, and walls of the sleeping apartments of man, it may be considered as indirectly parasitic. It usually emerges at night from its lodging for the purpose of securing its nourishment in the blood of its victims.

This parasite is reddish brown in color, oval in shape, from 4 to 5 millimeters in length and 3 millimeters in breadth. These insects, if crushed between slides or as more usual between the hand and a part of the victim's body, have a characteristic odor very much resembling kerosene. The blood is drawn from the victim by means of a long proboscis. The eggs are approximately 1.12 millimeters in length and require about eleven months for their development to the sexually ripe insect. These eggs are retained in the crevices of the bed, floors, furniture, wall-paper, and other parts of the dwelling, so that the complete removal of these eggs and parasites is a matter of some difficulty.

That these insects have more or less importance from the standpoint of transmission of disease from one person to another must be remembered. Individuals vary in their susceptibility to the bite of the bedbug, some being indifferent to it, while others are markedly affected by it.

Diptera.

Pulex Irritans (Common Flea).—The male is from 2 to 2.5 millimeters in length, the female as much as 4 millimeters. It is a red or brownish-red insect, having a laterally compressed body, an oral rostellum, serrated soft mandibles, a tongue sheathed in an inferior labium, and a pair of labial four-jointed palpi. Each of the triple segments of the thorax bears a pair of five-jointed double-clawed legs. The female deposits her eggs, not on the human being, fortunately, but in the fissures, crevices, or holes of garments or furniture which may be accessible.

Pulex Penetrans (Sand Flea, or Jigger).—This parasite is a minute, brownish-red, egg-shaped insect which penetrates the skin of man. The female is the infecting insect and produces painful irritation and even suppuration.

VEGETABLE PARASITES.

Achorion Schönleinii.—This organism is the cause of the disease known as favus or tinea favosa. This fungus invades the root sheaths, the bulbs, and the shafts of the hair filaments of the scalp, but it also occurs upon the "non-hairy" portions of the skin and upon the nails. The spores gain access to the

deeper layers of the skin and develop around the hair-shaft, forming a characteristic yellowish, cup-shaped crust which has a peculiar, mouse-like odor.

In searching for this parasite, a favus crust is softened by the addition of a few drops of water or dilute sodium hydrate solution and placed upon a slide and examined with the high-power dry lens. The hairs may also be examined in the same manner or may be stained by methods outlined in the discussion on *Tinea trichophytina.*



FIG. 32.—ACHORION SCHÖNLEINI.

The mycelial threads appear as narrow, flattened, ramifying, short or elongated, linear cells or tubes, which may be simple and empty, or be divided more or less regularly by transverse partition walls transforming the longer and simpler into shorter and compound cells (see Fig. 32). The latter often contain in their cavities sporules clinging to either side, in which case the mycelial threads are termed sporophores. The conidia are encapsulated or are strung together like the beads of a necklace, and appear as round, oval, angular, or very irregularly contoured bodies. These mycelial threads branch at right angles; the spores measure from 3 to 10 microns in diameter (Hyde).

Trichophyton Megalosporon Endothrix.—This organism is the cause of tinea circinata (herpes tonsurans, ringworm of the

body) and of tinea sycosis (tinea barbæ, ringworm of the beard, barbers' itch).

The trichophyton is composed of spores, which vary greatly in size, but which, as a rule, are somewhat larger than those of the type next to be discussed. They are frequently cuboidal, oval, or irregularly rounded, but their chief characteristic lies in their arrangement in lines or chains, extending up and down the hair shaft (see Fig. 33). The mycelium is found without, but never within, the hairs (Hyde).

These fungi may be stained by the method of Morris and Calhoun. The hair is first washed in ether to remove all fatty

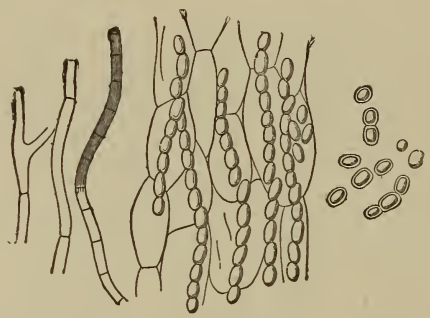


Fig. 33.—Trichophyton Spores and Threads.

débris; it is then put for one or two minutes in Gram's iodine solution and is stained after drying in gentian-violet for one to five minutes. It is again dried and treated for a minute or two with the iodine solution and for an equal length of time in aniline oil containing pure iodine, after which it is cleared with aniline oil, washed in xylol, and mounted in Canada balsam.

Microsporon Audouini (Trichophyton Microsporon).—This parasite appears under the microscope chiefly in the form of a large number of round spores, irregularly grouped or massed about the follicular portions of the hair. Mycelial threads, large and branching, are often seen within the hair. The sheath of spores surrounding the hair is often continued upward for $\frac{1}{16}$ to $\frac{1}{8}$ inch above its exit from the follicle and may be recognized as a whitish or grayish coating of the hair. These mycelial threads are all within the hair proper, thus differing from those of the trichophyton, which are never within the hair; after re-

peatedly dividing and subdividing, they terminate on the outer surface of the shaft in fine filaments, at the extremities of which are the spores. This parasite is the cause of tinea tonsurans, or ringworm of the scalp.

Microsporon Furfur.—This parasite is readily recognized by the microscopic examination of the scales scraped from the skin. Innumerable clustered spores, highly refractive and resembling in their circular and oval contours droplets of oil, are quite characteristic. The mycelial threads are not usually branched, but lie in a close network among which sporophores are distinguishable, with conidia and terminal elements emerging at one extremity of the spore case. Both elements of this organism are more readily stained by the aniline dyes than are those of the trichophyton or favus. This organism is the cause of the condition known as *tinea versicolor*.

VI.

DETERMINATION OF THE FUNCTIONS OF THE STOMACH.

THE GASTRIC CONTENTS.

The significance of the term gastric contents, in the following pages, is taken to mean the material found in the stomach and extracted by the gastric tube at the expiration of a fixed and definite period after the ingestion of a test-meal.

THE VOMITUS.

It is not good practice to utilize vomitus for the purpose of chemical analysis. Such material is usually of very uncertain composition, being contaminated with mucus from the upper part of the tract; further, the amount or composition of the food previously ingested is an unknown factor, affecting materially the quantitative and qualitative findings. However, it may be found of decided advantage to test all vomited matter for the presence or absence of acidity and free hydrochloric acid. In the presence of suspected cancer the vomited material may be searched for necrotic tissue shreds or sarcinæ.

METHODS OF OBTAINING SPECIMEN FOR EXAMINATION.

The usual apparatus employed to remove the test-meal comprises the well-known gastric tube of soft red rubber, fitted at one end with a soft rubber funnel, and containing near this a bulbous expansion without valves. A recent modification of and improvement over this is the large bulb devised by Ewald. This bulb is sufficiently large to contain the total quantity of material removed, thus overcoming in a measure the difficulties of the smaller bulb. These two methods are usually successful in obtaining the desired material for examination, but are difficult to manage and possess a decided disadvantage in that

they do not provide for the important procedure of lavage and inflation.

Also, tubes of irregular caliber are difficult to cleanse, and unless great care is exercised in this direction, they may become a source of contamination, if not carriers of infection.



FIG. 34.—COMPLETE OUTFIT FOR GASTRIC TEST-MEAL REMOVAL, LAVAGE AND INFLATION.

Some years ago Dr. Judson Daland adopted for this purpose two large open-mouth bottles and a double-action Davidson bulb; these are used in conjunction with the plain gastric tube. With this arrangement, aided by an assistant, it is possible to rapidly and cleanly obtain a sample of gastric contents (in con-

siderably less than a minute), and to follow immediately with lavage and inflation if desired.

To those who have used this method with uniform success in both private and hospital practice, its advantage is evident, the only drawback being the almost absolute necessity for trained assistance, this being occasioned by the complicated nature of the apparatus, which requires a second pair of hands to manipulate the bottle and its connections during the passage of the tube. To overcome this drawback the author has devised a reversing valve (see Fig. 34). With this valve it is unnecessary to make any change in the tube connections when once the apparatus is set up. The perfected device has been worked out with great care, having in mind the necessity of a simple mechanism capable of being cleansed and kept in repair without difficulty.

PRELIMINARY PREPARATION OF THE PATIENT.

The preliminaries leading up to the extraction of the test-meal should be as nearly uniform in every case as possible. By adopting a definite routine and adhering to it we eliminate, in a great measure, the errors which would otherwise creep in and lessen the value of the findings. The adoption of the following rules will enable the examiner to obtain a series of reports in one case or in groups of cases of far greater value than could be obtained by an irregular technic:—

First.—It is advisable that all medication should be withheld until after the gastric analysis has been made. If this is impossible, then a period of days should elapse before making the test, during which all treatment is stopped.

Second.—The test-meal should be given on an empty stomach, either one which has been emptied by fasting or by previous lavage.

Third.—The volume and composition of the test-meal should be uniform. The particular meal employed to be determined by the operator according to his preference.

Fourth.—The removal of the meal should (depending on its composition) be accurately timed; this time to be measured from the beginning and not from the end of the meal.

Fifth.—The test-meal should be removed without dilution if possible. If dilution should be necessary to accomplish re-

moval, the amount of water used should be definitely known, and the total withdrawn must exceed the amount used. If this rule is not observed, all investigations of a quantitative nature are valueless. For method of determining total volume of gastric contents, see page 181.

Sixth.—The tube should remain within the esophagus only long enough to allow time to compress the bulb. If much time elapses after introduction before removal of the contents, the hypersecretion of mucus occasioned by the presence of the tube will alter the composition of the sample and possibly result in erroneous conclusions.

A tube of relatively large diameter is to be preferred, being easier of insertion, less uncomfortable to the patient, and insuring greater success in obtaining a sample. A suitable tube should measure between ⅜ and ½ inch outside diameter, and should be provided with both a terminal and lateral opening in the gastric end.

A lubricant for the tip of the tube is unnecessary, the contact of the tube with the patient's pharynx immediately exciting sufficient flow of mucus for this purpose. This is preferable to either nauseating oil or hygroscopic glycerin. In case of great reflex excitability of the pharyngeal constrictors, this region may be first sprayed with a dilute solution of cocaine.

COMPOSITION OF THE USUAL TEST-MEALS.

(*a*) **Ewald Test-meal.**—This meal is, perhaps, the most frequently employed for general work. It consists of a roll, or piece of bread or toast (about 35 grams) without butter, and 2 cups of water or tea (about 300 cubic centimeters) without milk or sugar.[1]

The contents are removed one hour later. This amounts normally to 30 to 50 cubic centimeters, depending both upon the skill of the operator and upon the condition of the stomach. Hypermotility of the stomach will diminish the quantity of contents recovered, while a hypomotility will increase the quantity.

(*b*) **Modified Ewald Breakfast.**—Of the test-meals employed, one of generally useful composition includes the white

[1] It has been shown that it is almost impossible to detect blood in the presence of tannic and gallic acids, so that it is advisable not to use tea in the test-meal when blood is suspected.

of 2 eggs poached or soft boiled, without yelks and without seasoning; 2 pieces of toast without butter (the slightest trace of butter or fat will cause lactic and butyric acid fermentation), and a cup of tea without milk or sugar.

This meal should be removed at the expiration of one hour, when, under ordinary circumstances, there will be recovered between 30 and 90 cubic centimeters.

(*c*) **Boas Test-meal.**—This meal consists of a dish of oatmeal prepared by concentrating to 500 cubic centimeters a liter of water to which a tablespoonful of oatmeal is added. The chief advantage of this meal is that it introduces a digestible material that is free from lactic acid, which is a normal constituent of bread. The contents of the stomach are withdrawn one hour later when, owing to the small volume of material introduced, the amount may be very small. If the stomach shows normal digestive powers most of the material will be then passed into the intestines, while an appreciable amount of material recovered would indicate either a dilatation of the stomach or pyloric obstruction.

(*d*) **Riegel Test-meal.**—This test-meal has the advantage of permitting the patient to use a more normal diet than in the ones previously mentioned. This is of considerable importance in America, where we are not accustomed to the Continental breakfasts.

The Riegel meal is given in the middle of the day and consists of about 400 cubic centimeters of soup, 200 grams of beefsteak, and either 2 slices of white bread or 150 grams of mashed potato, together with one glass of water. This meal should be withdrawn at the expiration of three to four hours. The advantages of this meal are that they incite a more nearly normal gastric juice than the preceding ones, and give some indication of the rate and amount of the digestive process, but has the disadvantage that particles of undigested food frequently clog the tube and interfere with the removal of the contents.

(*e*) **Fischer's Test-meal.**—This meal, introduced by an American physician, has the advantage of more nearly approaching an American breakfast than the others. It consists of the bread and tea of the Ewald meal together with ¼ pound of finely chopped lean beef broiled and seasoned. The contents are re-

moved at the end of three hours. Fischer has shown, by comparing results after this meal with those of the Ewald breakfast, that with his combination the results are much more constant, though somewhat higher, than with the other Ewald or Boas breakfasts.

(*f*) **Salzer Test-meal.**—This is a double meal and is given as follows: For breakfast the patient receives 30 grams of lean cold roast meat, finely chopped; 250 cubic centimeters of milk; 60 grams of rice, and 1 soft-boiled egg. Four hours thereafter a second meal is given, consisting of 37 to 70 grams of stale wheat bread and 400 cubic centimeters of water. The contents are removed one hour after this second meal. Under normal conditions of digestion and motility the stomach contents should show no remnants of the first meal.

THE DALAND-FAUGHT TEST-MEAL APPARATUS.

The component parts of the apparatus are as follows:—

A plain gastric tube without bulb or funnel.

A double-action Davidson hand-bulb.

A large rubber stopper having two perforations.

Two wide-mouth bottles containing more than a liter each, and graduated in cubic centimeters.[2]

A short length of large glass-tubing and some ¼ inch rubber-tubing.

The reversing valve.

Technic of Removal.—Fill one graduated bottle to the 500 cubic centimeter mark with warm sterile water. Fit the double perforated stopper with the glass tube and the reversing valve. Place the stopper firmly in the empty bottle and attach the gastric tube to the glass tube, and the two ends of the double action bulb to the two horizontal tubes of the valve. Finally, ascertain the direction of the air-current through the valve by making a few pressures on the bulb. Set the valve to make negative pressure within the bottle.

The tube should now be passed, with the patient preferably in the sitting posture. As soon as the tube enters the cardiac

[2] These special bottles are not necessary for practical purposes; any large, open mouth bottle of sufficient quantity, such as a quart milk bottle, may be substituted. A mark must be made at the measured 500 cubic centimeter mark on one bottle.

orifice, a few quick pressures are made on the bulb. This develops a slight degree of negative pressure within the bottle, when the gastric contents will immediately flow into the bottle. Sudden stopping of the flow from occlusion of the tube by particles of food or mucus may immediately be removed by momentarily reversing the lever. This will cause a small portion of the gastric contents to return through the tube, effectually washing out the obstruction. This simple maneuver may be repeated as frequently as necessary to obtain a sufficient specimen.

In the event of failure to obtain sufficient material by this means, the difficulty will usually be overcome by the introduction of a measured amount of water. To accomplish this the stopper with all its connections is removed from the bottle and fitted into the bottle containing the 500 cubic centimeters of warm water. The valve is set to make positive pressure within the bottle, and by means of the bulb about 400 cubic centimeters are run into the stomach; then by reversing the valve the whole is withdrawn. It is necessary to recover more than a total of 500 cubic centimeters if any determinations of a quantitative nature are to be made.

If it has been necessary to resort to the introduction of water to effect the removal of the test-meal, allowance must then be made in the final calculations, so that the results will represent the quantities in pure gastric contents.

For Example: Suppose, after employing 500 cubic centimeters, a total of 550 cubic centimeters is recovered; of this amount only 50 cubic centimeters represent actual gastric contents, or 1 part in every 11. It will be necessary then to multiply any figures obtained in the calculations of acidity by the factor 11 in order to express the results in terms of undiluted gastric contents.

Türck's Double Stomach-tube.—F. B. Türck[3] has devised a double tube consisting of two single tubes placed parallel to each other; the smaller, inlet tube, has a diameter of 5 millimeters; the larger, outlet tube, has a diameter of 12 millimeters. The outlet tube is longer by 10 centimeters than the inlet tube, thereby allowing the larger tube to reach the floor of the stomach.

[3] Chicago Medical Record, December, 1907.

The contour of the two tubes fused together is that of a flattened oval, which conforms to the shape of the esophagus. This tube is said to be more flexible and therefore less uncomfortable to the patient than the regular tube and at the same time allows of a maximum outlet for the stomach contents and, because of the pressure exerted by the bulb, assures a maximum of successful removals.

Method of Use.—After the tube has been inserted to the proper distance, the large inflating bulb is placed on the upper end of the inlet tube. A sudden puff of air from the bulb will insure immediate expulsion of the stomach contents through the outlet tube. This apparatus may also be used for purposes of determining the gastric contour by inflation.

TO DETERMINE GASTRIC CONTOUR AND POSITION.

Inflation of the Stomach.—Next to the X-ray, probably the best method of determining the size, shape, and location of the stomach is by the introduction of air. To accomplish this two methods are available. Of these, the second is greatly to be preferred for reasons to be stated later:—

1. The first consists in administering 1 dram of sodium bicarbonate dissolved in a little water, to be immediately followed by an equal quantity of tartaric acid, also in solution. The combination of these causes the evolution of carbon dioxide gas within the stomach, which immediately distends that organ. This method is open to serious objection, because the quantity of gas produced cannot be controlled and overproduction, besides causing great discomfort if not doing actual damage, may result in hemorrhage and great cardiac embarrassment. On the other hand, sufficient gas may not be evolved to completely distend the stomach, and thus its full size and shape fail to be accurately determined. In the light of these facts it would seem best that this method be permanently abandoned for the following, which is more in accord with the principles of scientific medicine:—

2. The second method of inflation is accomplished through the stomach-tube by means of a Davidson bulb. This simple combination may be employed with safety and accuracy, and

even in the absence of the graduated bottles and reversing valve can be made to serve.

A greater refinement in the technic is attainable with the aid of bottles and valve above described. Their use may conveniently follow the removal of the test-meal. With this outfit in addition to inflation we may also roughly measure the cubic contents of the distended stomach, by introducing a measured quantity of air.

Technic of Inflation.—By inflation the position of the stomach may be accurately outlined. After the test-meal has been removed the patient is placed in the semi-recumbent posture, and the empty stomach is outlined as accurately as possible by auscultatory percussion. Air is then introduced into the stomach through the tube in sufficient quantity to produce a distinct change in the auscultatory percussion-note. The quantity of air required to accomplish this is quite small, not sufficient to alter the relation of the organ to surrounding viscera, as is the case when the stomach is ballooned with air. By the proper working of the valve and the bulb on the apparatus, we can change the gastric note at will, thereby being able to differentiate absolutely between gastric and colonic tympany.

By this method gastroptosis can be absolutely determined, the author having in many instances had the results confirmed by the X-ray.

Contraindications to Inflation.—These are the same as for the use of the stomach-tube itself, viz.: Myocardial degeneration, with or without endocarditis; angina pectoris, aneurism, advanced vascular degeneration, hemorrhage from any part of the body, and all diseases in which hemorrhage is likely to occur. Gastric ulcer is, of course, a contraindication, especially when hemorrhage has been noted. Another contraindication, and one which is not sufficiently emphasized, is neurasthenia and allied mental states with gastric symptoms. In these diseases the symptoms referable to the stomach are not due to organic change in that organ, but are psychic in origin. The use of the tube in these cases serves but to reinforce the idea of disease of the stomach, and renders a cure more difficult and occasionally impossible. This, of course, refers to those cases which are obviously psychic in origin. In certain cases it is not possible to

determine whether the disease is organic or not; here the tube would have to be used for diagnostic purposes. It has been suggested that the unpleasantness of the passage of the tube itself will act as a curative agent. Experience teaches that usually more harm than good will come from the use of the tube with this intent.

To Determine the Capacity of the Stomach by Inflation.—To accomplish this the apparatus employed in the preceding de-

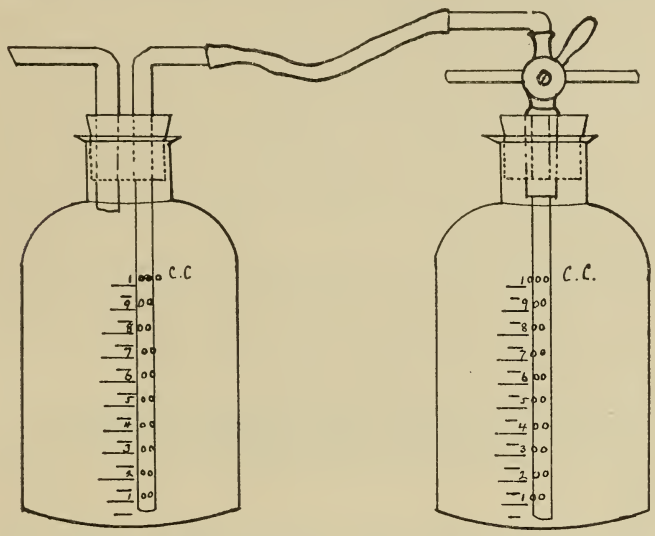


FIG. 35.—DIAGRAMMATIC REPRESENTATION OF ARRANGEMENT OF BOTTLES FOR MEASURING CUBIC CONTENTS OF STOMACH.

scription must be augmented by the addition of a second double-perforated rubber stopper and a second section of glass tubing, arranged in a similar manner to the first.

The apparatus is to be set up as follows (see Fig. 35): The extremity of the gastric tube is attached to the short glass tube of the second stopper, and a section of tubing made to join the long glass tube of each stopper. The bottle belonging to the valve and bulb is filled with water to the 1000 cubic centimeter mark, and both stoppers placed in their respective bottles. Now, with the tube in the stomach and the valve set for compression, the water is gradually forced over into the second bottle. This in turn displaces the air, and forces it into the stomach. When

the patient indicates that the stomach is full, the amount of water displaced from the first bottle will equal the amount of air forced into the stomach, and can be read in cubic centimeters from the scale on the bottle. (See diagram.) The valve should now be reversed and the air withdrawn.

Technic of Gastric Lavage.—Prepare a large pitcher of sterile water at body temperature, and a receptacle for waste. (If desired normal saline or dilute alkaline water may be substituted.) Place 500 cubic centimeters of the wash-solution in one bottle, and by means of the valve and bulb force a few hundred cubic centimeters into the stomach; then, after allowing it to remain for a few moments, reverse the valve and withdraw as much as possible. Discard the returned water and repeat this process until the wash-water returns clear. A quantity of this saline or alkaline solution may be allowed to remain when the tube is finally withdrawn.

Indication for the Use of the Gastric Tube.—The tube is positively indicated in cases of carcinoma associated with pyloric stenosis. The result here is decided relief from distressing symptoms, and often actual prolongation of life.

In certain cases of pernicious anemia, especially in the later stages, stagnation of food gives rise to distressing symptoms, and by allowing absorption of toxins hastens the progress of the disease.

Also, in cases where poison has been taken into the stomach in toxic doses, the stomach tube may be of great service in affording prompt removal.

Contraindications to passing the tube are: uncompensated heart disease of any kind, either muscular or valvular; aneurisms, advanced pulmonary tuberculosis, apoplexy, or recent and very severe hemorrhage from the stomach, especially in the case of ulcer or carcinoma of that organ. Menstruating and pregnant women should not be examined unless the diagnostic or therapeutic results expected are of great importance. Ulcer of the stomach is not an absolute contraindication, however, if great gentleness is used in passing the tube, and the stomach is not too much distended by fluid. The tube should be used only for important diagnostic or therapeutic purposes. It is safer to treat a suspected case as ulcer and avoid the passage of the tube.

THE CHEMISTRY OF DIGESTION.

GASTRIC ACIDS AND ACIDITY.

Gastric Contents from Fasting Stomach.—The stomach is practically never empty, but always contains a certain amount of acid fluid. Boas considers anything between 1 and 100 cubic centimeters as a normal amount of material for the fasting stomach. Anything above this amount would mean either motor insufficiency or hypersecretion. One may differentiate these two conditions by washing out the stomach at night, when the material withdrawn in the morning will be extremely scanty if the condition is one of motor insufficiency. Riegel regards any material in the fasting stomach as pathologic.

This fluid from the fasting stomach is thin, has a specific gravity of 1004 to 1005, contains some free hydrochloric acid, no lactic acid, and no bacteria. It is very commonly bile-stained, may be alkaline from the presence of pancreatic juice, and may contain large amounts of mucus. As such material is always found in the fasting stomach, it is well to make it a rule to wash out the stomach the night before giving a test-meal.

CHEMICAL COMPOSITION OF THE GASTRIC JUICE (containing water with saliva).

Water	994.40	parts.
Solids	5.60	"
Organic material	3.10	
Mineral salts	2.50	
Sodium chlorid	1.46	
Calcium chlorid	0.16	
Potassium chlorid	0.55	
Ammonium chlorid	Trace.	
Calcium phosphate	Trace.	
Magnesium phosphate	Trace.	
Iron	0.12	
Free HCl	0.20	

SECRETION OF HYDROCHLORIC ACID.

Under conditions of health, after from ten to fifteen minutes following the ingestion of food the gastric contents are acid, due to the presence of free acids or acid salts. At this time the free acid recognized is lactic acid. Up to thirty-five

or forty minutes lactic acid predominates, and only traces of HCl can be detected. Shortly after this the lactic acid disappears and only HCl remains, so that at the end of one hour no lactic acid can be demonstrated.

Hydrochloric acid is actually present from the beginning, but its presence is masked by the excess of lactic acid and the HCl combined with bases. Free HCl increases with the progress of digestion until it reaches 0.15 to 0.2 per cent. after a light meal, or from 0.2 to 0.33 per cent. after an abundant meal.

FREE ACIDS.

QUALITATIVE TESTS.

For the simple qualitative demonstration of free acids, organic and inorganic, the *Congo red* and *tropeolin papers* are convenient. The former turns dark blue, the latter dark brown, when moistened with a solution containing free acids, but neither of these react to acids which are combined with bases.

Detection of Free Hydrochloric Acid.—For the qualitative determination of the presence of free HCl, a number of tests are available.

Tests.—1. Töpfer's Dimethyl-amido-azobenzol: For this test either the 0.5 per cent. alcoholic solution of this chemical can be used, or for convenience filter-paper may be soaked in the 0.5 per cent. solution, allowed to dry, and then kept bottled for use. This solution is delicate for 0.003 per cent. HCl. Combined HCl as well as acid salts and inorganic acids, in the concentration in which they occur in the stomach, will not cause this solution or the prepared paper to become pink. A pink reaction denotes the presence of free hydrochloric acid.

2. Gunzberg's Phloroglucin Vanillin (for reagent see Appendix).—This reagent, if active, is of a pale-yellow color. It darkens and deteriorates on exposure to light; so should be kept in a dark-colored bottle or prepared fresh each time that it is required. A drop or two of this reagent is placed in a porcelain dish, together with an equal amount of filtered gastric contents, and heat gently applied until the liquid has evaporated. If HCl be present a rose-red color will appear at the margin of the evaporating fluid. This test is unmistakable and

is most delicate, demonstrating the presence of free HCl in the proportion of 0.05 per 1000. The reaction is not interfered with by albuminates, by salts present in the usual amount, or by organic acids.

3. Boas's Test.—This reagent consists of 5 grams of resorcin and 3 grams of cane sugar dissolved in 100 grams of 95 per cent. alcohol. It has the same delicacy as Gunzberg's test and is more stable. The test is applied in the same way as the preceding, taking particular care to use a low flame in evaporating, and it gives a rose-red or vermilion color in the presence of mineral acids. This color gradually fades on cooling and is not given by organic acids or acid salts.

4. Tropeolin Test.—The reagent for this test is a saturated alcoholic solution of tropeolin 00. This test is applied in the same way as the preceding and gives a lilac-blue color in the presence of free acid. This test is not as delicate as the preceding, reacting only in the presence of 0.3 part of free hydrochloric acid per thousand, and has the objection that it strikes the blue color much more easily with the organic acids.

5. Friedrich Test.—Friedrich[4] uses neither sound nor special apparatus. His method consists in producing the necessary chemical reaction directly in the stomach by means of threads saturated with Congo red. A small metal cylinder with rounded edges is enclosed in a capsule and attached to a long thread dyed with Congo red; another dyed thread is attached to the cylinder with one end left free and enclosed within the capsule. This capsule, with its enclosed metal cylinder, is swallowed readily by the patient, twenty minutes after the usual test-breakfast has been given. Half an hour later, it is withdrawn by means of the long thread when, according to the degree of the reaction on the thread, he estimates the amount of free hydrochloric acid. The author has had no difficulty in getting his patients to swallow this capsule and he has repeatedly verified his findings by means of other tests. It should be mentioned here that the long thread is dyed a deep red, whereas the short thread is dyed pink, so that the degree of reaction may be noted. The author has laid down the following scale of the reaction for himself; browning of the dark-red thread indicates subnormal

[4] Berlin. klin. Wochen., July 22, xlix, No. 30.

acid; violet, normal; blue-black, above normal or hyperchlorhydria. In the presence of hyperchlorhydria, the pink thread is colored blue if the condition is of high grade, and sky blue if the hyperchlorhydria is extremely intense. The reaction must be noted immediately when the thread is withdrawn and before it is touched with fingers or instruments.

QUANTITATIVE ESTIMATION OF TOTAL ACIDITY.

The reaction of the filtered gastric juice being determined by the Congo red or the tropeolin papers, the total acidity is next determined by titrating against a decinormal sodium hydrate solution.

Technic.—A Mohr burette is filled to the "0" mark with standardized decinormal sodium hydrate solution.

To 10 cubic centimeters of filtered gastric juice in a porcelain dish, 10 cubic centimeters of distilled water is added, and 1 or 2 drops of a 1 per cent. alcoholic solution of phenolphthalein added as an indicator. (This indicator is inactive in the presence of carbon dioxid.) In the presence of free acids this mixture is colorless. The sodium hydrate is now run in slowly with constant stirring, until the rose color, which appears on the addition of each drop of alkaline solution, no longer disappears nor is intensified by further addition of the sodium hydroxid solution.

As a rule, the acidity of the gastric contents, one hour after the ingestion of the test-breakfast, requires from 4 to 6 cubic centimeters of decinormal NaOH to neutralize 10 cubic centimeters of gastric contents. For example, suppose 5.2 cubic centimeters were required to neutralize 10 cubic centimeters of gastric contents (this is within normal limits). The result of the estimation is usually expressed in parts of decinormal NaOH per 100 parts of gastric filtrate. This expression is easily obtained by moving the decimal point one place to the right. The result in the above example would, therefore, be recorded as 52 parts of decinormal NaOH per 100 parts of gastric contents.

QUANTITATIVE ESTIMATION OF FREE HYDROCHLORIC ACID.

To 10 cubic centimeters of filtered gastric juice add an equal quantity of distilled water, and 1 or 2 drops of Töpfer's

reagent. This mixture, placed in a porcelain dish, is titrated with a decinormal solution of NaOH until the pink color of the solution has been entirely removed and is replaced by pale yellow. Suppose, for example, 6 cubic centimeters were the amount required to neutralize the free HCl contained in 10 cubic centimeters of gastric contents. To obtain the percentage of HCl in the gastric contents the following calculations are required:—

One cubic centimeter of decinormal NaOH solution is equivalent to 0.00365 gram of HCl (decinormal NaOH is equivalent to 4 grams of NaOH dissolved in exactly 1000 cubic centimeters of distilled water; each 1 cubic centimeter of this solution should exactly neutralize 0.00365 gram HCl). Therefore, 6 x 10 would equal the number of cubic centimeters of NaOH solution required for every 100 cubic centimeters of gastric contents, and the result, 60 x 0.00365, would equal the percentage of HCl in the specimen under examination, which would be 0.219 per cent. HCl.

DETERMINATION OF THE TOTAL AMOUNT OF GASTRIC JUICE SECRETED.

In order to determine the total amount of gastric juice secreted, a somewhat complicated procedure is necessary. It is evident that measurement of the quantity removed does not give us the total secretion, as it is impossible to obtain the last few cubic centimeters from the stomach.

By the method of Mathieu and Remons as much of the gastric contents as possible is withdrawn into one graduated bottle and set aside. A measured quantity (300 or 400 cubic centimeters) of water is then run into the stomach from the second bottle, allowed to remain for a few minutes, and is then withdrawn into the second bottle. If b is the quantity of gastric juice obtained by direct removal before the addition of water, a the total acidity of this undiluted juice, c the acidity of the diluted gastric juice, and q the amount of water added to the stomach, then the acidities a and c are inversely as the quantity of water used, since the greater the amount of the wash-water the less the total acidity of the second fluid withdrawn.

This may be expressed by the following formula:—

$$a : c = q + x : x$$

From which we derive $x = \frac{c\,q}{a - c}$

The amount of gastric juice originally present in the stomach is therefore $b + \frac{c\,q}{a - c}$

This formula assumes that acid is present in the stomach contents. In diseases where no free acid is present, sufficient $\frac{n}{10}$ acid may be given by the tube to cause an acid reaction in the stomach before proceeding, by which the combined acid may be estimated.

Causes of Lowered Gastric Secretion.—I. In acute and chronic gastric inflammation.

II. Atrophy of the gastric mucosa from any cause.

III. General functional depression.

IV. Expression of a gastric neurosis.

V. Congenital idiosyncrasy.

DETERMINATION OF ORGANIC ACIDS.

These include lactic and acetic, and the true fatty acids, particularly butyric. Acetic and fatty acids are not found during normal digestion, and if present, as they sometimes are, have either been introduced with the food or have been produced by fermentation of carbohydrates set up in the stomach by bacteria introduced with the saliva.

The physiologic presence of lactic acid during the first stage of gastric digestion may be due either to its formation within the stomach, or from its having been introduced with the food, as in baked bread.

Uffleman's Test for Lactic Acid.—The addition of a few drops of filtered gastric contents to Uffleman's reagent (see Appendix) in a test-tube will, in the presence of lactic acid, change the amethyst-blue to a canary-yellow. This test is positive in the presence of 1 part of lactic acid in 20,000.

Sources of Error.—Lactates cause the same reaction. This is, however, immaterial, since we desire to recognize lactic acid,

whether free or combined. The reaction also takes place with alcohol, sugar, and certain salts, particularly the phosphates, but rarely in their usual concentration after the test-breakfast.

Strauss's Method for Lactic Acid.—This method is, perhaps, the very best clinical method at our disposal, as it shows

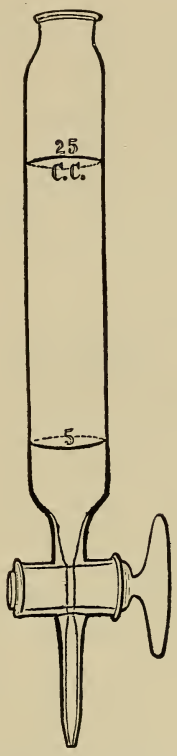


Fig. 36.—Strauss's Separatory Funnel.

lactic acid when present in pathologic amounts. It does not, however, give a quantitative result, nor does this seem necessary in the ordinary clinical work. Into a special separatory funnel (see Fig. 36) are introduced 5 cubic centimeters of the gastric juice. The funnel is then filled to the 25 cubic centimeter mark with alcohol-free ether and well shaken. The ethereal layer will take up the lactic acid from the gastric contents. After the fluids have settled, the gastric juice and ether are allowed to

run out to the mark 5 by opening the stop-cock, after which distilled water is added to make up the 25 cubic centimeter volume. Two drops of 10 per cent. ferric chlorid solution are then added with a medicine dropper and the mixture well shaken. The water will now extract the lactic acid from the ether. The aqueous layer is colored an intense greenish yellow if more than 0.1 per cent. of lactic acid is present, while smaller amounts will show a slight greenish tinge. This test may be negative if the lactic acid present is completely combined with the proteins of the gastric juice. In such cases hydrochloric acid may be added to liberate this lactic acid before shaking out with ether.

The fatty acids, particularly butyric, strike a tawny-yellow color with a reddish tinge with Uffleman's reagent. The reaction is positive with 1 part in 2000. Fatty acids may also be detected by heating to boiling a few cubic centimeters of gastric filtrate in a test-tube over the mouth of which a strip of moist neutral litmus paper is placed. On this the vaporized volatile acid will produce the usual change.

Butyric Acid (Pineapple Test).—If a portion of the dried ethereal extract of the gastric juice (see method of Strauss above) be treated with a few drops of concentrated sulphuric acid and a little alcohol, the odor of ethyl butyrate is perceptible on slight warming. This odor is the peculiar one of pineapples and is very easily recognized.

Acetic acid is easily recognized by its odor, but it may also be detected by neutralizing a watery residue after the removal of an ethereal extract, with sodium carbonate, and then adding neutral ferric chlorid solution. In the presence of acetic acid a striking blood-red color will be produced. This neutralization of the aqueous solution is an essential point in this test, as the presence of free acid will prevent the appearance of any precipitate, and the presence of free alkali will cause the formation of ferric hydroxid, which will mislead, as the coloration is very much the same. A similar reaction occurs in the presence of formic acid, but this acid is never present in gastric juice.

Alcohol.—This is sometimes formed in the stomach during intense yeast fermentation. This may be detected by the *Lieben's iodoform test,* applied as follows: To a portion of the

gastric filtrate add a small portion of liquor potassii, and then a few drops of a solution of iodine and potassium iodide in water. (See Appendix.) If alcohol be present a yellowish scum gradually occurs on the surface, which is readily recognized as iodoform by its odor. The same reaction occurs in the presence of acetone, but occurs more rapidly.

Propeptone and Peptone.—These are products of albumin digestion, and when present indicate the activity of this part of the gastric function. A few drops of filtrate added to hot Fehling's solution produce a purplish color, if they are present.

Starch.—The addition of a drop of Lugol's solution to a piece of filter paper upon which some unfiltered gastric contents has been allowed to fall will, in the presence of starch, yield a blue reaction. This reaction is intensified by the addition of a drop of crude nitric acid.

MICROSCOPIC EXAMINATION OF GASTRIC CONTENTS.

The examination of the gastric contents would not be complete without an examination of the sediment, both in an unstained wet preparation and a stained dry specimen.

For this, a small portion of the unfiltered material should be placed upon a clean slide and covered by a large cover-glass; and examined by the low and medium powers, when much confirmatory information may be obtained.

Under normal conditions, various budding forms of yeast cells will be seen; also food remains, the character of which will, of course, be determined by the nature of the test-meal and the presence or absence of gastric retention and fermentation. Starch cells will be recognized by their irregularly oval oyster-shell forms, with eccentric striations. Meat fibers and muscle cells will show the characteristic striations, which will become more indefinite as the process of peptic digestion progresses. Elastic fibers will have their characteristic fibrillated, wavy appearance.

Vegetable and fruit cells have their own peculiar appearance, which will be easily recognized. Occasional epithelial cells are encountered, and granular indeterminate *débris* goes to make up the bulk of the solid material present. This contains

a varying amount of non-pathologic bacteria. Stagnant or decomposed specimens will show a larger number of yeast cells and crystals of calcium oxalate, tyrosin, cholesterin, etc.

In pathologic conditions red blood-cells and pus-cells may be recognized, and more rarely tissue fragments, which by appropriate methods of staining may be identified as portions of carcinoma or other tumor. Occasionally flagellates, amebas, and other monads may be seen.

The specimen employed above may be used for staining. By sliding the cover-glass off, a very fair smear will be made, which after drying and fixing may be stained by any of the simple dyes.

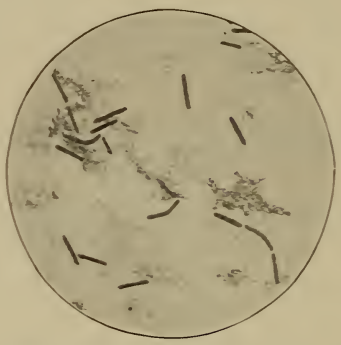


FIG. 37.—BOAS-OPPLER BACILLUS IN GASTRIC CONTENTS.

Examination of the stained specimen with the $\frac{1}{12}$ objective will show innumerable micro-organisms, among which may be mentioned *sarcinæ ventriculi,* which are usually considered evidence of dilatation and fermentation, but which are not themselves pathogenic.

The Boas-Oppler Bacillus.—This organism is found quite commonly in patients suffering with carcinoma of the stomach, and rarely in non-malignant disease. It is found more frequently in the gastric contents at a time when lactic acid is present in large amounts, so that in the incipient stages of carcinoma these organisms may be absent. These bacilli are very large (3 to 10 microns by 1 micron) and are frequently joined end to end, forming very long chains (see Fig. 37). They are readily stained with the usual aniline dyes and by Gram's method. On being treated with the dilute iodin, they take on a

brown color, which distinguishes them from the large mouth bacillus (*Leptothrix buccalis*), which stains blue with iodine.

SPECIAL EXAMINATION OF STOMACH FOR LEUKOCYTES.

Robertson and others have demonstrated the close relation of leukocytes in the washed stomach contents to gastric carcinoma. This examination is made by first carrying out a thorough gastric lavage and then using the last clear water for the examination. A portion of this water is centrifuged and the sediment smeared on a slide, dried, and stained. The presence of leukocytes in this preparation is said to be almost conclusive evidence of gastric carcinoma.

TESTS FOR OCCULT BLOOD.

Blood may be intermittently present in the gastric contents or gastric vomitus as a result of one of the following conditions:

I. Ulcer of the stomach or intestines.

II. Benign pyloric stenosis.

III. Spasm of the pylorus.

Occult blood is usually constantly present in cases of malignant disease of the stomach or esophagus.

Boas Modification of the Weber Test.—To 15 cubic centimeters of gastric contents, add an equal amount of ether and thoroughly agitate. To this mixture add 3 to 5 cubic centimeters of strong glacial acetic acid, and again agitate. Now allow the mixture to settle and decant 10 or 15 cubic centimeters of the clear supernatant liquid, and to this add 4 or 5 cubic centimeters of ozonized oil of turpentine. In the presence of blood a violet or blue color will appear, which is intensified by the addition of chloroform. (See also test on page 209.)

Acetic Acid Ether-Guaiac Test.—To 10 cubic centimeters of gastric contents add 10 cubic centimeters of ether and 5 cubic centimeters of strong acetic acid. Thoroughly shake, and add 2 or 3 grains of powdered gum guaiac, and again agitate; allow to settle, and then add a few drops of fresh solution of hydrogen dioxide. In the presence of blood a purple or blue ring will appear at the line of contact, or the solution grad-

ually assumes a grayish-blue color. (See Appendix for phenolphthalein test.)

ESTIMATION OF PEPTIC ACTIVITY.

The digestive power of the filtered gastric contents from the recovered test-meal depends upon, first, the amount of pepsin contained, and, second, upon the amount of free acid present, particularly the free hydrochloric acid. Artificial digestion is the only means at our command by which we may determine the peptic activity of the gastric juice.

Method of Ewald.—Prepare from the albumin of eggs, which have been boiled just sufficiently to cause firm coagulation, small discs or squares by first cutting thin slices of the coagulated albumin, and from these slices the cubes or discs. These bits of albumin may be prepared in bulk and preserved in glycerin, which is carefully washed off before using.

The Test.—Place an equal quantity (5 to 8 cubic centimeters) of filtered gastric juice in each of four test-tubes, add to each tube one or two pieces of the prepared albumin; then,

To tube 1 add nothing.

To tube 2 add 1 drop of HCl.

To tube 3 add 4 grains of pepsin.

To tube 4 add the above quantities of HCl and pepsin.

These tubes are now placed in an incubator at 37° C., and from time to time examined to note the progress of liquefaction of the albumin. By comparison we can roughly determine whether digestion is progressing normally in the unaltered tube, and whether pepsin, hydrochloric acid, or both are necessary to accomplish it.

The results obtained from this investigation can only be considered in the light of an approximate indication of the peptic activity of the material tested.

According to Nierenstein and Schiff,[5] it is nesessary to differentiate three factors in order to arrive at an adequate idea of the significance of these pepsin determinations. These are: the diluting secretion, the pepsin secretion, and the hydrochloric acid secretion, all three of which must be regarded as distinct expressions of the secretory activity of the glandular parenchyma.

[5] Arch. f. Verdauungskrankh., vol. iii, 1902.

Following a better knowledge of the activity of these different factors, two improved modifications of the method of pepsin determination have been advanced. These are the methods of Hammerschlag and of Mett.[6]

Method of Hammerschlag.—Briefly outlined, the method depends upon the activity of a few centimeters of gastric juice upon a 1 per cent. filtered solution of egg albumin. Two test-tubes, one filled with the albumin solution alone and the other with the albumin solution plus the gastric filtrate, are incubated for an hour at 37° C. At the expiration of this time the albumin content of each tube is estimated volumetrically, according to the method of Esbach (see page 264). The difference between the albumin precipitate in the two tubes is equal to the amount of albumin digested, and, therefore, is a measure of the peptic activity of the gastric juice. The objections to this method are that the Esbach method is not very accurate, and also that albumoses are partly precipitated by the reagent. However, this method is sufficiently accurate for the ordinary clinical purposes, and when the Mett method cannot be followed may be adopted. It would be better, however, to employ a method which employs diluted gastric juice, as in the Mett method.

Mett's Method of Peptic Determination.—Glass capillary tubes from 1 to 2 millimeter[7] diameter and of convenient length are filled by suction with the fluid portion of egg albumin. In order to avoid accidental variation in the egg albumin, it is better to use the albumin of several eggs mixed. These tubes as they are filled should have their ends plugged with bread crumbs to prevent loss of contents before coagulation. After filling they are placed horizontally in a simmering water bath, where they are allowed to remain for from four to five minutes. While boiling, the tubes should be kept in motion to ensure uniform coagulation of the albumin. When freshly made, the albumin tubes contain innumerable bubbles, which, however, gradually disappear; after four or five days the tubes are ready for use. After boiling, the tubes are wiped dry and the ends closed with sealing wax or stick lac to prevent drying. Thus prepared a stock of tubes may be kept indefinitely, but it

[6] Sahli's: "Diagnosis," 1907.
[7] J. A. D., Petersburg, 1889, from Paulow's Laboratory.

should be ascertained before using that the albumin is still evenly in contact with the walls of the tube; if they have dried out they are not fit for use.

These tubes are cut as needed into lengths of from 2 to 3 centimeters. This cutting is best accomplished by nicking the glass with a small triangular file, when a quick bend at this point will usually cause accurate fracture of both tube and albumin. Portions containing air bubbles or showing irregular fracture should be discarded.

Technic.[8]—For quantitative determinations of the peptic activity of the filtered gastric contents, employ a dilution of 1 to 16, using as a diluent $\frac{n}{10}$ HCl, using for each test 1 cubic centimeter of contents and 15 cubic centimeters twentieth normal HCl.

This mixture is placed in a covered Stender dish[9] after two Mett tubes have been placed in it, and the specimen allowed to digest in an incubator (37° C.) for twenty-four hours. At the end of this period the digested cylinders of egg albumin at each of the four ends are measured off and the average reading calculated. The relative amount of pepsin is then obtained by squaring the result (Shurtz's law), and if desired multiplying by the dilution, *e.g.*, 16. For making the measurements a pair of calipers with a vernier reading 0.1 millimeter is convenient. A lens is useful principally for reading the vernier. One end of the tube is placed against one jaw of the calipers, and the other is separated until its end is just visible through the opalescent edge of albumin. If the tube is at all oblique the shortest side is taken, while if the albumin is at all uneven, the highest point to which digestion has extended is taken. In place of the calipers the ordinary mechanical stage, which is usually fitted with a vernier scale, may be used.

Nierenstein and Schiff have proven that a dilution of at least 1 to 16 is absolutely necessary if we wish to obtain a relative idea of the quantity of pepsin as estimated from the digestion length. This is because with a dilution of less than 16, the length of digestion decreases as the length of time increases,

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[8] After Sailer and Farr, U. of Pa. Med. Bull., Oct., 1906.

[9] A Stender dish is similar to a Petri dish, but is deeper, with a flat ground-glass lid.

owing to the concentration and activity of the inhibiting substances. This great dilution also decreases the amount of pepsin in the mixture, so that the digestion length is kept within the limits of Shurtz's law (3.6 millimeters in twenty-four hours). As there are instances when this dilution is not sufficient (with very active pepsin solution), it may become necessary, when the digestion length exceeds 3.6 millimeters with the 16-fold dilution, to repeat the pepsin test with a dilution of 1:32; then by squaring the length as before, and multiplying this quantity by 32, the relative amount of pepsin in the undiluted gastric contents is obtained. It is clear that in this estimation the unit of the relative amount of pepsin will be that quantity of pepsin by which 1 millimeter of albumin in the Mett tube will be digested in twenty-four hours with an acidity of 0.18 HCl. In this determination we do not consider the absolute quantity of pepsin, but simply the degree of concentration, for the result of Mett's method is the same whether large or small quantities of digestive substances are employed.[10]

Summary.—Conclusions of Sailer and Farr regarding variations due to alterations in technic[11]:—

1. Difference in the caliber of the tubes. The variations in the reading of the two ends of one tube were as great as between tubes of different caliber for tubes between 1 and 2 millimeters. In tubes greater than 2 millimeters the rate of digestion seemed to be uniformly greater in tubes of larger diameter.

2. The age of the tubes. Provided there is no separation of the albumin nor putrefactive softening, age does not seem to affect the tubes. The method of preparation tends to make them sterile. Tubes that are too fresh (less than four or five days old) are said to digest more rapidly than tubes that are "ripe."

3. Variability in the digestion of albumin from different eggs is an unimportant factor.

4. The degree of digestion within certain limits is said to vary directly with the duration of digestion.

5. Variations in the temperature of the incubator. This is undoubtedly an important factor, but there are no definite data to offer.

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[10] Sahli's "Diagnosis," fourth edition, 1905.
[11] Sailer and Farr: *Loc. cit.*

6. The effect of variations in the acidity of the original specimen. This has been provided against in the technic of the method, since the use of so large an amount of diluting acid solution tends to render the acidity of the diluted specimen uniform in every instance.

GASTRIC EXAMINATION.

Determination of Pancreatic Activity.—The determination of pancreatic activity by the oil test-meal is based upon the experimental work of Boldiroff, who found that the introduction of oil into the stomach of dogs caused regurgitation of the duodenal contents in which pancreatic ferments could be demonstrated. Volhard devised a method of demonstrating trypsin and applied it to clinical uses.

This method has recently attracted sufficient attention to warrant a brief consideration of the method at this time, although the findings have not yet been proven conclusive.

The *clinical application* as advocated by C. B. Farr[12] is as follows:—

From 100 to 200 cubic centimeters of olive oil or cotton-seed oil are administered through the stomach-tube, into the fasting stomach. Food-remains or fluid if found in the stomach should first be removed through the tube.

At the expiration of half an hour the contents of the stomach is aspirated; at this time there is usually recovered only a few cubic centimeters of a whitish mucoid fluid; in others as large an amount of pale green or dark green fluid is obtained.

The tests are carried out upon the fluid without filtration.

Reliable material for the trypsin test is not always forthcoming, as, while practically always some fluid is recovered, frequently this is nothing more than a small amount of retained or freshly secreted gastric juice or mucus.

Farr suggests that the best criterion as to the character of the fluid should be its color, indicating the presence or absence of bile, and its response to the action toward ferments. I would suggest that the reaction would be also of great value in determining the origin of the fluid.

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[12] Jour. A. M. A., Dec. 11, 1909.

Methods.—*The Melt Method* (fully described on page 189): This may be successfully employed, the only modification being the substitution of a decinormal solution of sodium carbonate for the HCl as described; particles of fibrin are placed in the dishes to detect tryptic digestion.

Method of Gross.—A 0.1 per cent. solution of casein in a 0.1 per cent. sodium carbonate solution is prepared and 10 cubic centimeters placed in each of several test-tubes. The trypsin solution serially diluted with water is added in 1 cubic centimeter amount to the several tubes and all are incubated at 37° C. for twenty-four hours. At the end of this time 1 per cent. acetic acid is added to each of the tubes and the dilution noted in the tube in which cloudiness last appears. If the casein is completely digested no cloud appears.

Quantitative Method.—Up to this time no practical method of determining the amount of trypsin present in a given specimen has been found, so that we are only to say whether trypsin is present or absent.

The Clinical Application.—There is yet not sufficient clinical data at hand from which to draw any conclusive findings. Lewinski[13] and others believe that the absence of trypsin after this procedure shows either pancreatic insufficiency or pyloric spasm. This may be true but the evidence is as yet insufficient. It would be safer to say that the presence of trypsin in the oil test-meal would exclude any disease involving to a marked degree the activity of the pancreatic gland.

ESTIMATION OF THE ACTIVITY OF RENNIN OR MILK-CURDLING FERMENT.

Normal gastric juice contains, besides hydrochloric acid and pepsin, the rennin ferment as a natural secretory product of the gastric mucosa. Rennin possesses the property of coagulating milk without the presence or assistance of acids.

Method of Leo.—To 10 cubic centimeters of fresh, uncooked neutral or amphoteric milk, add from 2 to 5 drops of filtered gastric juice, and place the mixture in an incubator at 37° C. If rennin is present in normal amount, curdling should take place in from ten to fifteen minutes. In this process the

[13] Lewinski: Deutsche med. Wochen., Sept. 10, 1908.

slight amount of acid contained in the gastric filtrate is insufficient to cause coagulation. If curdling takes place very slowly it is questionable whether this change is due to the action of the rennin or to the formation of lactic acid, so to be exact the reaction of the mixture should be taken before and after curdling has occurred. The rennin reaction is certain to have occurred only in the presence of an unchanged reaction. If coagulation does not occur within an hour rennin can be considered absent. As a further guide it may be remembered that the characteristic curd from rennin is a cake of casein floating on clear serum, while the curd from lactic acid is lumpy and broken.

DIGESTION OF STARCH AND SUGAR.

During digestion starch is converted into grape-sugar, while cane-sugar is converted into invert-sugar (a mixture of cane- and grape- sugar).

Starch is recognized by the deep blue color produced by the addition of a dilute solution of iodine or Lugol's solution. This reaction grows less vivid as the starch is converted. If starch digestion is unduly delayed or does not occur, we may infer hyperacidity of the gastric juice.

DETERMINATION OF THE RATE OF ABSORPTION FROM THE STOMACH.

Penzoldt's Method.—A capsule containing potassium iodide (1½ grains) is swallowed. The appearance of the iodine reaction in the saliva indicates that absorption has occurred from the stomach. To test for the iodide in the saliva, paper moistened with starch paste and dried is used. After the capsule has been swallowed, the paper is moistened with saliva at short regular intervals, and then touched with a glass rod dipped in commercial (better, fuming) nitric acid. Upon the appearance of iodine in the saliva the characteristic blue reaction occurs.

When absorption is normal this reaction is usually positive in from ten to fifteen minutes; but if absorption is delayed, the reaction may be slow in appearing or occur not at all.

DETECTION OF BILE.

A New Reaction for Bile Pigments in Gastric Contents.—A. V. Torday and A. Klier[14] in working with the stomach-contents of a jaundiced patient found that the methyl-violet, with which they were testing for free hydrochloric acid, gave a red color instead of the usual blue. The urine of a jaundiced patient gave the same reaction. Investigating further, they found that various staining fluids gave similar reactions with the bile-stained urine. Pure bile pigments have not been studied. They suggest the following:—

TEST.—The method consists of adding 1 drop of a 1 per cent. solution of the dye to 15 cubic centimeters of water, and to this 1 cubic centimeter of gastric filtrate. According to these observers, the delicacy of these tests was found to be about twice as great as the iodine or the Gmelin test.

TEST OF THE MOTOR FUNCTION OF THE STOMACH.

If attempted extraction of a full meal six hours after ingestion fails when properly performed, or if nothing can be recovered from an Ewald test-breakfast after two and one-half hours, the motor function of the stomach may be considered normal.

A SECOND METHOD is to administer a large dose (10 or 15 grains) of salol and test the urine at definite periods for the appearance of the products of its decomposition. The components of salol, carbolic and salicylic acid, are separated in the alkaline juice of the small intestine. They remain unchanged and undissolved during gastric digestion. Salicylic acid is readily detected in the urine by the violet color produced by the addition of neutral ferric chlorid solution. This test is conveniently performed by moistening filter-paper and bringing a drop of ferric chlorid solution in contact with it. If gastric peristalsis is normal, salicylic acid should begin to appear in the urine from forty to sixty or seventy-five minutes after ingestion of 15 grains of salol.

IODOFORM METHOD.—Give with the test-breakfast 1 gram of iodoform in a well-sealed capsule. The iodoform, being in-

[14] Med. Record, Oct. 2, 1909.

soluble in the gastric juice, will not be absorbed by the stomach, but will be passed on by peristaltic action to the intestine. By the action of the fluids of the duodenum the iodoform is decomposed with the formation of soluble sodium iodid. The demonstration of the iodid in the saliva by means of starch paper and nitrous acid will indicate the time when the gastric contents is being discharged into the intestine. Iodin should normally be detected in the saliva in from one hour to one hour and a half after the ingestion of the capsule.

Boas's Method.—Boas administers a simple evening meal consisting of meat, bread and butter, and tea, washing out the stomach the following morning. If any food material is found the motor insufficiency is considerable. If the stomach be washed out previous to the administration of the evening meal, no food should be found in the stomach in the morning.

INDIRECT EXAMINATION OF THE STOMACH CONTENTS.

Gunzburg's Method.—A tablet of 0.2 gram of potassium iodid is placed in a piece of the thinnest possible strongly vulcanized rubber tubing measuring about 2.5 centimeters in length. The ends of the tubing are folded and the package tied with 3 threads of fibrin which have been hardened in alcohol. The package is now tested by placing it in warm water for several hours and examining the water for potassium iodid. The patient swallows one of these packages three-fourths of an hour after an Ewald meal, the saliva being tested for potassium iodid at intervals of fifteen minutes. In the presence of free hydrochloric acid, in normal amounts, the threads of fibrin are dissolved and the potassium iodid is absorbed, giving a reaction in the saliva in from one to one and three-fourths hours. In cases of hypochlorhydria the reaction is delayed for six hours, indicating a practical absence of free hydrochloric acid.

Sahli's Desmoid Reaction.—Sahli has recently introduced the "desmoid bag" for use in estimating the functional activity of the stomach. These bags are made of the ordinary rubber dam used by dentists and contain a pill of 0.05 gram of methylene-blue and 0.1 gram of iodoform. The bag is tied, in a manner specially outlined by Sahli, with catgut which has been

allowed to dry, but which has been untreated chemically. This gut, according to Sahli, is digested only by the gastric juice and not by the pancreatic juice. This pill is administered to the patient immediately following the noon meal and the urine and saliva tested at intervals of one hour, beginning three hours after administration of the pill. The digestion of the gut by the gastric juice liberates the pill and permits of the absorption of both the methylene-blue and the iodoform. The methylene-blue will appear in the urine, coloring it green within six hours, while the iodine will be found in the saliva within two hours. Should the color of the urine not be distinctly green, this tint may be more clearly brought out by adding a few drops of acetic acid and boiling. Variations from the periods indicated above denote a hyperacidity or a hypoacidity of the gastric juice according as the time of appearance of the reactions is shortened or increased. As the gut is digested only by the gastric juice, a non-appearance of either reaction would indicate an anachlorhydria.

RÖNTGEN RAY EXAMINATION.

With the advent of the Röntgen ray and its practical application, it has become an invaluable aid to diagnosis. A number of competent men have applied this agent in the study of the digestive tract, and have reached so many valuable conclusions pertaining to the size, location, motility, etc., of the stomach that the examination of a patient suffering with any disorder of the digestive tract must be considered incomplete unless a röntgenologic examination has been made. The procedure and technic are omitted from this work, because the examination is beyond the field of the general practitioner, requiring, as it does, special technic, expensive apparatus, and a large experience in röntgenologic interpretations.

VII.

THE FECES.

PHYSICAL CHARACTERISTICS.

The Number.—One stool per day is the normal average for a healthy adult. Three daily, or one in forty-eight hours, may be normal for some individuals, and not incompatible with health.

The Reaction.—Whether the stools be acid or alkaline is of no special clinical importance. In adults the reaction is usually alkaline, sometimes neutral, but rarely acid. Acid stools are the rule in infants.

The Amount.—The amount varies in proportion to the amount of solids ingested. A preponderance of vegetable food usually produces a large quantity, while animal food leaves comparatively little residue.

The average daily amount of feces varies between 60 and 250 grams, of which 75 per cent. is water.

To weigh solid feces ascertain the weight of both the feces and their container, then weigh the container empty, and subtract the latter from the former weight, which will represent the weight of the contained feces. If the feces are liquid they may be measured, and the amount expressed in cubic centimeters.

The Odor.—The disagreeable odor is largely due to the presence of indol and skatol, but may be further increased by hydrogen sulphide, methane, and methyl-mercaptan.

The Consistence.—This varies greatly and depends largely upon the amount of fluids ingested, the temperature, the climate, and the condition of the digestive tract. In man the usual form is the characteristic plastic cylinder. Clinically expressed, the consistence of the feces may be liquid, mushy, or solid or formed.

Serous Stools.—These are composed of fluid without fecal matter, and are of considerable diagnostic importance. Such

stools are characteristic of Asiatic cholera, cholera morbus, cholera infantum, and poisoning by antimony. In cancer of the rectum evacuations are small, frequent, and serous. Arsenic poisoning and acute catarrhal enteritis may produce this form of stool. Poisoning by toad-stools is also a frequent cause of serous stools.

The Color.—The color varies with the character of the food ingested, and is usually little influenced by the decomposition products of the biliary pigments. In adults, the color usually varies from a light to a dark-brown. Lack of color in the stools may occur in health, owing to the over-oxidation of the coloring matter into colorless products of bilirubin. Such stools do not necessarily indicate the presence of large amounts of fat nor obstruction of the biliary passages.

In infants, fat and undigested milk produce a whitish-color tinged with bile-pigments.

GREEN STOOLS are seen after taking calomel. After exposure to the air such stools are usually acid.

YELLOW STOOLS may be caused by the ingestion of santonin, rhubarb, or senna. Typhoid stools are yellowish-brown and have received the name of "pea-soup stools."

WHITE or CLAY-COLORED STOOLS frequently denote the absence of bile, as in obstructive jaundice.

Very dark or BLACK STOOLS may result from the administration of iron, manganese or bismuth, or from the ingestion of much meat, blackberries or red-wine.

"TARRY" STOOLS usually denote hemorrhage.

EXAMINATION OF INTESTINAL DIGESTION BY MEANS OF GLUTOID CAPSULES.

Sahli recommends the use of glutoid capsules which are made from gelatin hardened in formaldehyde. These either do not dissolve in the gastric juice at all, or only after a considerable time, but are rather quickly soluble in the normal intestinal juices.[1] These are preferable to the keratin coated pills which were originally recommended, and are useful to diagnose the condition of intestinal digestion, *e.g.*, the pancreatic func-

[1] Deutch. med. Woch., No. 1, 1897.

tion. Capsules are filled with some drug which does not diffuse through the capsule wall, and whose absorption may be studied by its appearance in the saliva and the urine. For diagnostic purposes glutoid capsules, containing 2 grains (0.13 gram) of iodoform and 4 grains (0.26) gram) salol, are convenient.

In order to make the conditions of the test as uniform as possible both as to the length of time the capsules remain in the stomach and the degree of digestive absorption, it is advisable to administer the capsule with the test-breakfast. Experience has shown that normally, under ideal conditions, *i.e.*, normal gastric motility, normal intestinal digestion, and normal intestinal absorption, the iodin reaction may be expected to appear in the saliva, and salicyluric reaction in the urine in from four to six hours.

If one wishes to test the accuracy of any particular lot of capsules, it will be necessary to prove that this reaction time is obtained in healthy normal individuals. Only rough approximate differences in the time of the reaction are of clinical importance, so that it is sufficient to examine the saliva and the urine after six, ten, and twenty-four hours. The best results from this method are obtained by administering the capsule in the morning upon an empty stomach, and then, four hours later, allowing the patient to resume his meals as usual. The specimens of urine and saliva may, of course, be saved, and examined afterward.

DETERMINATION OF THE MOTOR FUNCTIONS OF THE GASTRO-INTESTINAL TRACT.

Method of Adolph Schmidt.[2]—Schmidt demands two conditions for a satisfactory clinical method of examining the feces: 1. A knowledge of what a normal stool should be under a certain diet, involving the use of a "test-diet." 2. The methods of examination must be so simple that they are within the reach of every physician.

The Test-diet.—The requirements are: (*a*) That it be nutritious enough to furnish calories sufficient for the body's needs. (*b*) That it be so constituted that it can be readily ob-

[2] Quoted by Steele, Medical News, Dec., 1905.

tained in any household or hospital dietary. (*c*) That it contains a known amount of its constituents, so that variations in digestion and absorption can be detected in the stool.

Schmidt suggests the diet which is given below, followed by the approximate equivalents as suggested by Steele:—

Milk, 1.5 liters (2¾ pints).

Zwieback, 100 grams (3 ounces) well-dried toast.

Two eggs.

Butter, 50 grams (1½ ounces).

Beef, very rare or raw, 125 grams (¼ pound).

Potatoes, 190 grams (6 ounces).

Gruel made from 60 grams oatmeal (2½ ounces).

Sugar, 20 grams (½ ounce).

This may be given somewhat as follows:—

Breakfast.—Two eggs, half of the amount of toast and butter, 2 glasses of milk, oatmeal, and sugar.

Dinner.—The steak and potatoes, ⅓ of the amount of toast and butter, 1½ glasses of milk.

Supper.—Two glasses of milk, remainder of toast and butter.

This diet is sufficient for the needs of the average patient, and, while not offering much variety, is only taken for two or three days. A capsule containing 5 grains of charcoal is given with the first meal, and no examination made until the charcoal (black) appears in the stool.

The amount of each article composing the day's dietary must first be accurately determined and compared to the contents of convenient measures, so that the articles need not be weighed every meal. This does not apply to the meat and potatoes, which should be weighed each day.

The Period of Passage.—The period of time required for food to pass through the intestinal tract can easily be determined by watching for the black in the stool. It is quite as necessary to know the time required for the passage of chyme through the gastro-intestinal tract as it is to ascertain how often the stools occur, since the two are in no way identical.

It has been shown in chronic colitis with several watery stools a day that the period of passage may be normal, and that peristalsis is decidedly increased only in the colon. According

to Strass, using a diet similar to Schmidt, the time required for food to pass through the alimentary canal varies normally between ten and thirty hours. Under abnormal conditions this may vary between four and forty-eight hours.

The Specimen.—After the appearance of the charcoal, which indicates the beginning of the passage of the test-diet, a portion of one passage is collected in a clean receptacle and transferred to a clean, wide-mouthed bottle, and conveyed to the laboratory for examination.

Much can be determined from a naked-eye examination of such a mass. The technic for this examination, as outlined by Steele, is as follows:—

Take a piece of formed stool about the size of an English walnut or an approximate equivalent of liquid feces, and rub it up in a mortar with distilled water until quite smooth and liquid. Part of this is then poured into a Petri dish and examined in a good light on a dark background. A little experience will enable the observer to acquire a large amount of information about the composition of the stool by this macroscopic examination.

The Gross Appearance of Normal Stools.—In normal digestion nothing should be seen by the naked eye, save a varying number of brown points (oatmeal husks), cellulose and indigestible parts of food, and occasionally sago-like grains that look like mucus, but which are found upon microscopic examination to be grains of potato. If, on the addition of the water, fat globules are seen, they must be considered pathologic unless the patient is known to have ingested excessive amounts of fats, as olive or castor oil.

Gross Appearance of Pathologic Stools.—(a) *Mucus* in large or small flakes is not affected by rubbing in the mortar. The mucus appears as glassy, translucent flakes often stained yellow by bile-pigment. Doubtful cases may be decided by the microscope.

(b) *Pus* in sufficient quantity to be recognized macroscopically will have the characteristics of pus in any other region. It usually appears as small, yellowish streaks or collections throughout the stool.

(c) *Blood* in appreciable amount, if from high in the digestive tube, will color the stool dark reddish brown or black; becoming more nearly the color of fresh blood as the source of origin approaches the lower end of the bowel.

(d) *Parasites.*—Segments of tape-worm, also thread- and seat-worms, may be detected in varying numbers in patients so afflicted.

(e) *Foreign bodies* having entered the digestive tract, if indigestible and not impeded in their progress, may be recovered from the stool at varying periods after ingestion.

(f) *Calculi* from the various glands situated along the digestive tract, or from the intestinal canal itself (enteroliths), may be found in the feces, and appear as calcareous masses of various sizes and composition, depending upon their origin and age.

(g) *Remnants of connective tissue* and sinew from beefsteak. These can be detected by their whitish-yellow color and toughness, by which they can be distinguished from mucus. In case of doubt the piece should be examined microscopically with a drop of acetic acid. Connective tissue then loses its fibrous structure, while mucus becomes more thread-like. Small, single pieces of connective tissue can be found in normal stools.

(h) *Remnants of Muscle-fiber.*—These appear as small reddish-brown threads or small, irregular lumps.

(i) *Remnants of Potato.*—These appear like boiled grains of tapioca and may easily be confused with mucus. The microscope will show the true nature of these bodies.

(i) *Remnants of Potato.*—These appear like boiled grains and can be recognized by their shape and by their solubility in all acids.

Microscopic Appearance of Normal Stools.[3]—Three slides are prepared from the liquid feces. The first is merely a drop of the material to be examined by both low and high power. The second slide is prepared by mixing a drop of acetic acid and a drop of the liquid feces upon a slide, heating it to boiling and then putting on a cover-glass. The third slide is prepared by mixing a drop of liquid feces with a drop of Lugol's solution.

[3] Technic of E. Dutton Steele, Medical News, Dec. 16, 1905.

Microscopic Examination of Slides.—*Slide* 1.—will show (*a*) single small muscle fibers colored yellow with cross striations. Visible with a Leitz 3, but showing better with higher power. (*b*) Small and large yellow crystals of salts of the fatty acids. (*c*) Colorless (gray) particles of soap. (*d*) Single potato cells without distinguishable contents. (*e*) Particles of oatmeal and grain husks.

Slide 2.—A general idea of the fat-content of the stool may be obtained. Upon cooling, small drops of fatty acids may be found covering the whole preparation. The large crystals of salts of the fatty acids are broken up by the acetic acid, and fatty acids liberated. If the slide is heated again and examined hot, the fatty acids will be found to run together in drops, which, as the slide cools, break suddenly apart.

Slide 3.—Here should be found violet-blue grains in some of the potato cells, and small single blue points, probably fungi or spores.

Pathologic Microscopic Findings.—*Slide* 1. (*a*) Muscle fibers in excess, perhaps with yellow nuclei. (*b*) Neutral fat drops, or fatty acid, in soap crystals. (*c*) An excess of potato cells with more or less well preserved contents. (*d*) Parasite eggs, mucus, connective tissue, pus, etc.

Slide 2.—Fatty-acid droplets in excess.

Slide 3.—Blue starch grains in the potato cells or free oatmeal cells, fungi, spores or mycelia.

Formed Elements in the Feces.—(a) *Blood.* Red cells, if recently shed, may be distinguished by their characteristic form, or if disintegrated, only masses of brownish-red amorphous hematoidin will be found. In a certain percentage of cases characteristic crystals of hematoidin will be found.

(b) *Epithelial Cells.*—These are normally present in moderate numbers and represent the natural desquamation from the intestinal canal. They are more or less disintegrated, depending to some extent upon their height of origin in the intestinal canal, and upon the length of time they have remained free in the digestive tube. In catarrhal conditions they may be present in very large numbers, when they may assume diagnostic importance.

(c) *Pus Cells.*—These rapidly undergo decomposition, so that even when numerous and coming from a comparatively short distance above the rectum, they may be beyond recognition. The characteristic pus cell appears as a small round or slightly oval granular body. The presence of very much pus in the stools is indicative of rupture of an abscess into the intestinal tract.

METHOD FOR MICROSCOPIC EXAMINATION OF EXTRACTS OF FECES.

Smithies[4] describes a procedure, recently adopted at the Mayo clinic, intended to increase the value of the routine microscopic examination of feces. A 2 per cent. agar jelly is first made by boiling strip agar in distilled water and filtering several times while very hot. The product is sterilized and kept for convenience in test tubes each containing 5 cubic centimeters. In examining a specimen of feces emulsion, the agar jelly is liquefied by heating, 2 cubic centimeters of it are poured into each of two small test-tubes, and 15 drops of filtered staining agent then added. For staining bacteria, epithelia, etc., Unna's polychrome methylene-blue is used; for starch elements and vegetable fibers, Lugol's solution. Thin smears of the feces are made on coverslips, dried, covered with 1 drop of the agar stain mixture, and mounted on slides. As the agar cools it solidifies, while the stain mixed with it permeates the smear. The agar gives so firm a mount that the specimens may be examined with high power and the stage of the microscope at any angle. The mount is sufficiently permanent to allow of future study of the specimens. By using the agar without any stain, motile micro-organisms may be observed for a long time.

CHEMICAL EXAMINATION.

This comprises only five routine tests: 1. The *reaction.* 2. The *sublimate* test for the condition of the bile salts. 3. The *fermentation test.* 4. The test for "lost albumin." 5. The test for occult blood.

1. The Reaction.—This is quite difficult to get with the ordinary litmus paper. It can be easily determined by dropping

[4] Archives of Internal Medicine, June, 1912.

a little softened fecal matter into 5 or 10 cubic centimeters of weak watery solution of neutral litmus, shaking it and noticing the color reaction.

2. The Sublimate Test.—This consists of taking a few cubic centimeters of liquid feces and mixing with an equal amount of a saturated watery solution of $HgCl_2$. A normal stool will quickly turn a pinkish red, indicating the presence of hydrobilirubin, which will be more intense the fresher the material. A green color is pathologic and indicates the presence of unchanged bile-pigment.

3. Fermentation Test.—Described by Steele, using his modification of Strasburger's apparatus.

The apparatus consists of a 2-ounce, wide-mouth bottle A (see Fig. 38). This is fitted with a perforated cork through which runs a tube to the test-tube B, which is also fitted with a rubber cork with two perforations. A bent tube runs from the tube B to the test-tube C, to allow for the escape of air. Each tube has a capacity of a little more than 30 cubic centimeters when fitted on the corks. The apparatus is simple and easily constructed, and broken parts can be replaced readily.

The Test.—About 5 grams of solid feces, or an equivalent of liquid feces, are rubbed up with a little distilled water and placed in the main bottle A. This is filled with sterile water, the tube is filled with water and fitted into place (not necessarily full), and tube C is then fitted on empty. The reaction is carefully noted before the test is started. The apparatus is then stood in a warm place—best in an incubator at 37° C. for twenty-four hours. If gas forms by fermentation in A it will rise into B, and the amount will be indicated by the amount of water displaced into C. Normally the fermentation test should show practically no gas, and the original reaction of the material should be unaltered in twenty-four hours. If more than one-third of the tube C is filled, it is pathologic. If the reaction after twenty-four hours is decidedly more acid, it is a carbohydrate fermentation. If alkaline, and with a foul smell, it is a fermentation of the albumins.

4. Test for Lost Albumin.—A portion of softened stool is filtered (a slow and difficult process). The filtrate is shaken with silicon and refiltered, then is saturated with acetic acid to

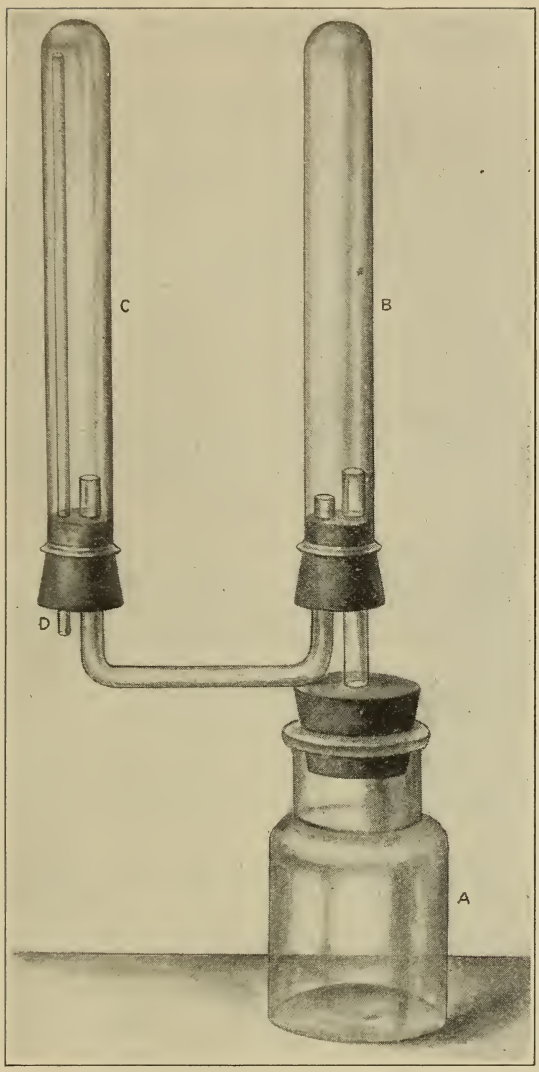


FIG. 38.—STRASBURGER APPARATUS, SHOWING ARRANGEMENT OF BOTTLES FOR FERMENTATION TEST OF FECES. (AFTER STEELE.)

bring down the nucleo-proteids, and finally a drop of potassium ferrocyanide solution is added. A decided precipitate indicates albumin. A positive test shows only that there is a decided diminution in albumin digestion.

BLOOD IN THE STOOL.

If bleeding has its origin in the upper part of the digestive tract (stomach or small intestine), it is so altered by the action of the digestive fluid that, by the time it finally appears in the stool, it has a black or brownish-black appearance which has been likened to tar or coffee grounds. However, if the hemorrhage be large and the peristalsis very active, blood may appear almost unchanged in the stool, even when of high origin in the digestive tube.

Relatively small amounts of blood may be so changed and mixed with the feces that they cannot be detected by the naked eye or by the microscope. This is termed "occult" blood, and is only recognizable by chemical means.

Preliminary Technic.—Owing to the possibility of the presence of a positive reaction, resulting from the ingestion of hemoglobin-containing food in a normal individual, it becomes necessary to restrict the diet in order to eliminate this possible source of error, and to limit the test-diet by the administration of a capsule containing 5 or 10 grains of charcoal, and to watch the stools for the appearance of the black discoloration due to the passage of the charcoal. Only after the appearance of the charcoal in the feces should a specimen be taken for the test for occult blood.

Steele[5] recommends a "liquid diet," including milk and broths, or a "semi-liquid diet" composed of milk, eggs, and toast, to which may be added moderate amounts of the ordinary winter vegetables.

Red meats and beef juice should positively be withheld. Steele has shown that iron, either in the organic or inorganic form, will even in large doses not affect the reaction.

As a preliminary to the test, it is necessary to eliminate as fully as possible extraneous sources of blood. Thus, tuber-

[5] Amer. Jour. Med. Sci., July, 1905.

culous ulcer, typhoid fever, hemorrhoids, fissure, and purpura should be excluded. Also ingestion of carmine, swallowed blood from any cause, hemoptysis, epistaxis, and menstruation.

Technic of Test.[6]—If the feces are solid they must be softened with distilled water. To 5 or 6 cubic centimeters of liquid feces, in a wide-mouthed cork-stoppered bottle, add about thrice as much ether and agitate or thoroughly mix by shaking. Then add a few grains of powdered guaiac and again agitate. Follow this by 5 cubic centimeters of glacial acetic acid (99.4 per cent.), and still again agitate. Allow this mixture to stand until the solid particles settle to the bottom, and then decant into each of two test-tubes 5 cubic centimeters of the supernatant liquid.

One test-tube should be kept as a control. To the other add 1 or 2 cubic centimeters of a fresh solution of hydrogen dioxid. If a bluish discoloration occurs either at the line of contact of the two solutions or throughout the mixture, the reaction is positive.

Klunge's Aloin Test.—According to Boas, the guaiac test for blood does not always yield decisive results in the examination of the feces, since the blue color is often veiled by brown shades and rendered indistinct. For this reason a control test with aloin, as suggested by Klunge and others, is desirable. This test is performed as follows: 5 cubic centimeters of the stool are extracted with 20 cubic centimeters of ether, in order to remove the fat, which would subsequently interfere with the test by the formation of emulsions. After the removal of the ether 3 to 5 cubic centimeters of acetic acid are added to the feces, and the mixture is again extracted with ether in a test-tube. The acetic acid-ethereal extract obtained in this manner is then employed for the investigation. A solution of aloin is prepared by dissolving as much aloin as can be placed upon the point of a small knife in from 3 to 5 cubic centimeters of 60 to 70 per cent. alcohol. To the acetic acid-ethereal extract is first added 20 to 30 drops of a resinous oil of turpentine, and then 10 to 15 drops of the solution of aloin. If the stool contains blood the resulting mixture soon becomes bright red, and upon standing for a time

[6] Technic employed in Dr. J. Daland's Laboratory.

assumes a cherry color. If no blood be present the aloin solution remains yellow for at least one or two hours, and then acquires a slightly reddish tinge. According to Boas, the aloin reaction may be markedly accelerated by the addition of a few drops of chloroform. With this modification, agitation of the mixture results in the formation of reddish droplets, which settle in the bottom of the tube as an intense red precipitate. According to Brandberg, the oil of turpentine may be replaced by a dilute solution of hydrogen peroxide.

Benzidin Test for Blood.—A few granules of benzidin are dissolved in 2 cubic centimeters of glacial acetic acid. A small fragment of the stool is mixed with 2 cubic centimeters of water and boiled in a test-tube. Ten drops of the benzidin-acetic acid solution and 3 cubic centimeters of 3 per cent. hydrogen peroxid are mixed in a test-tube and a few drops of the cooked emulsion of feces then added. A greenish or bluish color shows the presence of blood. The ethereal extract of the previous test may also be used, adding the benzidin-acetic acid and peroxid.

This reaction is extremely sensitive and care should be taken to see that the patient is not eating meat. The benzidin may be dried on a paper, as suggested by Einhorn, and the boiled stool mixed with a little peroxid and dropped on the paper. The blue color should appear within two minutes.

R. F. Ruttan and R. H. M. Hardisty[7] say that in orthotolidin we have a reagent for occult blood that is more satisfactory than benzidin on account of its greater delicacy, its more lasting color, and the fact that it can be made into a solution that retains its delicacy unimpaired for three or four weeks. The substance known as tolidin, or orthotolidin, is a crystalline, basic body of the aromatic series with melting point from 120° to 130° C., very soluble in water, easily soluble in alcohol and ether, and closely allied to toluidin and benzidin. They assert that it is superior to the reagents in general use for clinical work. While benzidin is equally good for the detection of blood in the feces and stomach contents, this is true only for freshly prepared solutions of benzidin, solutions of which lose 50 per cent. of their delicacy in twenty-four hours, while tolidin remains unchanged

[7] Canadian Medical Association Journal, November, 1912.

for three or four weeks. Another point in favor of tolidin is that when the blood is in small quantity the reaction increases gradually in intensity and persists longer than with the other reagents.

(For the phenolphthalin test, see Appendix, page 420.)

Significance of the Occult Blood.—According to Boas, occult blood is constantly present in carcinoma of the gastro-intestinal tract; intermittently present in gastric and duodenal ulcers; occasionally in stenosis of the pylorus, and is absent in gastritis, hyperchlorhydria, and in the gastric neuroses.

BACTERIA AND PROTOZOA IN THE FECES.

There are many varieties of bacteria in the feces. They have been estimated to amount to about one-third of dried feces. Some are harmless at all times; others, which are, under ordinary conditions, harmless, may develop pathogenicity under certain circumstances.

The digestive tract of the human organism is, at birth, supposed to be free from bacterial life; almost immediately, however, organisms gain entrance and rapidly multiply. There is every probability that they, by virtue of certain chemical substances, which they elaborate, are an aid to the digestive processes. Thus we know that certain groups of organisms have the power to liquefy gelatin, others to digest proteid, etc.; even the mass of the bacteria, by their mere presence, are of benefit to the digestive tract, since they furnish bulk to the intestinal contents, and so aid in the downward movement of digested or partly digested material.

Bacillus Coli Communis.—This organism is constantly present in the feces, and is normally non-pathogenic. It has, however, been found in pure culture in cases of appendicitis, empyema of the gall-bladder, pyelitis, and cystitis.

Morphology.[8]—It is a rod with rounded ends, sometimes so short as to appear almost spherical, while again it is seen with

[8] Abbott's "Bacteriology."

very much longer threads. It may occur as single cells or joined together in pairs, end to end. It is motile, and does not form spores. It stains with the ordinary alkaline dyes, and is decolorized by Gram's method.

Compared to the typhoid bacillus, it will be found to be less motile; grows more rapidly on gelatine colonies, and luxuriantly on potato (typhoid growth usually invisible). Colon bacillus coagulates milk in incubator from thirty-six to seventy-two hours; typhoid does not. Colon bacillus decomposes sugar solutions; typhoid does not.[9]

Bacillus Typhosis.—This is a distinctly pathologic bacterium, and is the specific cause of typhoid fever. Its appearance in the stools is scanty, but usually can be isolated by appropriate methods during the first few days of the disease.

Pratt, Peabody, and Long[10] obtained it from the stools of only 31 per cent. of febrile cases, and none in twenty-one convalescents examined by them. They state that it occurs most in the blood, and that it does not develop in the intestinal contents except under unusual conditions. The bacillus in the intestines comes chiefly from the gall-bladder; frequently it is found in the urine in larger numbers than in the feces, and is found in greatest numbers in feces containing blood.

Morphology.—The bacillus is about three times as long as it is broad, with rounded ends. Its length may vary greatly, but its width remains fairly constant. It is actively motile, and Loeffler's method of staining will show it is possessed of many delicate flagelli (see methods of staining).

Its growth on potato usually is invisible. It does not cause coagulation of milk (colon bacillus does). Owing to the tendency to retraction of the protoplasm from the cell-envelope, and the consequent production of vacuoles in the bacilli, the staining is often irregular. It stains with the ordinary aniline dyes.

Bacillus lactis aërogenes and the bacillus **proteus vulgaris,** are probably always present in the stools. They are probably pathologic in many cases of cholera infantum.

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[9] For methods of plating, isolating, and culture, see works on bacteriology.
[10] Jour. Amer. Med. Assoc., Sept. 7, 1907.

Streptococcus aërogenes.—This is an etiologic factor in certain cases of entero-colitis.

Bacillus Dysentericus (Shiga bacillus) is the cause of one form of infantile dysentery, and is generally present in the discharges of such patients showing blood and mucus.

The Comma Bacillus.—This is the infective agent in Asiatic or true cholera.

Morphology.—It is a slightly curved rod of a length of from one-half to two-thirds that of the tubercle bacillus, but thicker. Its curve is never very marked, it may be nearly straight. It is a flagellated organism, but has only one flagellum attached to one end. It is actively motile. It does not form spores.

Cultural Characteristics.—On gelatine plates at room-temperature, its development can be observed after as short a period as twelve hours. It rapidly liquefies gelatine. It is strictly aërobic. It takes the ordinary stains.

Diagnostic Method.—A smear is made from one of the small, slimy particles found in the semi-fluid evacuations, dried, fixed, and stained in the ordinary way. If upon microscopic examination only curved rods, or curved rods greatly in excess of all other organisms, are found, the diagnosis of Asiatic cholera most probably is correct.

Tubercle Bacillus.—This organism probably is always present in all cases of intestinal tuberculosis.

Diagnostic Technic (R. C. Rosenberger's).—A selection of "any part of feces is made, there being no effort at a selection of any particular mass or portion. If the stool is solid a small mass is mixed with sterile water. After drying and fixing, the spread is stained with carbol fuchsin for fifteen or twenty minutes, cold. The excess of stain is drained off and Pappenheim's solution (see Appendix) is applied, and when the preparation is the color of the counter stain (methylene-blue) thorough washing in distilled water is resorted to, the spread being then dried and mounted in balsam. The most important point in the technic is to obtain a spread the color of the counter stain with not a particle of the carbol-fuchsin showing to the naked eye."[11]

[11] Solis-Cohen: New York Med. Jour., Aug. 21, 1907.

It is alleged, but not conclusively determined, that no other acid- or alcohol-fast bacilli will withstand this method excepting the tubercle bacillus.

Ameba Dysenteriæ.—This is now generally conceded to be the specific cause of the so-called amebic dysentery. In typical cases the stools contain much blood-stained mucus, containing large numbers of these amebæ. These parasites so resemble epithelial cells that a positive diagnosis can only be reached when they are seen under the microscope to move and to extend their pseudopods. To keep them alive sufficiently long for examination, the feces must be caught in a warm pan and a portion immediately transferred to a warm microscope slide for examination. The organism is from 8 to 50 microns in diameter (see Plate V). To develop the nucleus the organisms should be obtained fresh; they are killed by the addition of a few drops of acetic acid or corrosive sublimate (sat. watery solution) while on the microscope stage. The nucleus is spherical and about 5 microns in diameter (see page 145).

For intestinal worms, ova, etc., see section on "Animal Parasites."

THE CLINICAL SIGNIFICANCE OF THE EXAMINATIONS.

Mucus.—As a rule, the appearance of mucus in the stool indicates the presence of inflammation of the mucous membrane, and is the one trustworthy sign of that condition (Steele). There are two conditions in which mucus has no significance: 1. When thin mucus spreads over the surface of a hard stool. 2. The so-called mucus-colitis with the discharge of mucus casts.

Bile-Pigment.—A green color of part or all the stool (by the sublimate test) is pathologic, except in children. It means too short a period of passage through the intestines. A fresh normal stool should give a pink reaction with $HgCl_2$. If a color reaction of any kind is absent it indicates either absence of bile from the intestine, a very fatty stool, or the reduction of hydrobilirubin (urobilin) into leucohydrobilirubin, a colorless product (Steele).

Fat.—It will require some practice in the use of the diet to tell whether there is an excess of fat in the stool or not. As the

normal amount of fat varies within wide limits in normal feces, only a great excess can be considered abnormal.

Remnants of Meat.—Normally there should be only microscopic particles of connective tissue and muscle fiber. An excess of either is often visible to the naked eye.

Excess of connective tissue means insufficient gastric digestion, because fibrous tissue is digested in the juices of the stomach only. Excess of undigested muscle-fiber means disturbance in intestinal digestion, and probably means trouble in the upper part of the small intestine, as follows: *(a)* Indicating a total lesion of the pancreas and absence of proteolytic and tryptic digestion. *(b)* Trypsin may be present, but its activating enzyme, enterokinase, may be absent. *(c)* The period of passage of the food through the intestine may be so rapid that no time is given for its digestion. *(d)* Large pieces of undigested muscle may be present, because gastric digestion is imperfect and the connective-tissue framework of the meat has not been properly removed.

Pathologic carbohydrate fermentation means poor starch digestion and indicates, as a rule, disturbance in the small intestine, which is usually due to insufficiency in the succus entericus.

Pathologic albumin fermentation means a large remainder of albumin in the feces, and indicates, in Schmidt's experience, serious trouble, usually some anatomic change in the mucous membrane of the small intestine (Steele).

FOREIGN BODIES, CALCULI, AND CONCRETIONS.

An attack of biliary colic or gall-stones may be followed by the appearance, from time to time, of gall-stones in the feces, or they may appear without the previous occurrence of any symptoms referable to the liver or its appendages. In searching for them, the feces should be rendered thoroughly fluid by rubbing in water and then by passage through a sieve. Various forms of simple and complicated apparatus are upon the market, and are known as *stool-sieves*. A very satisfactory home-made substitute is made by filling in a wire-frame with two or three layers of gauze or cheese-cloth, the wire-frame being arranged to fit around the rim of the bowl of the closet. To be certain of

not missing the stones it is necessary to examine all movements for at least fourteen days following the cessation of the attack of colic. Even a careful and prolonged search may fail to find the concretion in certain undoubted cases of gall-stone disease. Sometimes because the stone causing the symptoms was not passed, but after being jammed in the neck of the bladder finally returned into the cavity of the gall-bladder itself. Again, the stone may be retained for a long time in a fold or diverticulum of the intestinal tract, and finally the concretion may have fallen to pieces in the intestine. Another explanation of why the stones fail to appear is that typical attacks of colic may be due to inflammation without the presence of gall-stones.

Gall-stones are concretions which form in the biliary passages. They vary in size from a pin-head to that of a pigeon's egg or even larger. They are composed chiefly of cholesterin and calcium-bilirubin in varying proportions; besides these there are present minute amounts of other bile-oxidation products and calcium-carbonate. A predominance of cholesterin produces a light-, and of calcium-bilirubin a dark-colored stone, the absolute color varying usually between a light- or dark-brown to a dark-olive green. Stones vary greatly in hardness, and on cross-section usually show distinct concentric layers of crystalline substance, sometimes of different colors. The surfaces may present a beautiful faceted formation from attrition between a number of stones lying in the bladder, or they may be irregularly granular.

It is important not to confound other intestinal concretions with gall-stones. Woody bits of food, particularly the cores of pears, have been termed pseudogall-stones. The microscopic examination of bits of these scraped off with a knife, present the picture of characteristic wood cells. Chemical examination will also prevent mistake (see below).

So-called biliary sand, in most instances, consists of these small pseudogall-stones. The existence of true biliary sand has not yet been conclusively proven. Another kind of pseudogall-stone consists of balls of fat and fatty soaps which are not easily melted. They are found in the stools after the administration of large amounts of olive oil, as in a favorite method of treating cholelithiasis.

For *chemical examination* gall-stones are at first dried, then powdered and treated with alcoholic ether to extract the cholesterin. This may then easily be recognized by allowing the extract to evaporate upon a watch-glass, when the characteristic glistening rhomboids of cholesterin crystallize out, and may be easily recognized by the microscope. After extraction with alcohol and ether the residue is treated, while cold, with very dilute potassium hydroxid. If the powder contains calcium-bilirubin, a yellow solution will be obtained which gives Gmellin's reaction.

The much rarer PANCREATIC CONCRETIONS differ from gall-stones in that they contain no bile-coloring matter, and are composed chiefly of calcium-carbonate, which dissolves readily with effervescence in hydrochloric acid.

Intestinal Stones or Fecal Concretions.—These are supposed to play an important part in exciting attacks of appendicitis, but seldom appear in the stools. They consist almost exclusively of ammonio-magnesium phosphate (triple phosphate), and should be examined after the manner of urinary calculi (see page 300).[12]

[12] Sahli's "Diagnosis."

VIII.

THE URINE.

PART I.

GENERAL CONSIDERATIONS.

The normal constituents of the urine are usually tested for by quantitative methods, since we are concerned with the actual amount of these substances and not with their presence or absence from a given sample. In testing for abnormal constituents, on the other hand, we are concerned, as a rule, with their presence or absence, though in certain instances we may desire to know the absolute quantity of these abnormal substances.

The Sample.—If we are to make a qualitative examination for abnormal constituents in a single sample of urine, it is best to collect a specimen about three hours after the ingestion of a hearty meal (dinner), as such a sample is most likely to contain the substances sought (usually albumin or sugar).

Cases which at times show a trace of albumin will usually show an increase late in the day or after active exercise, and the collection of specimens should be timed to meet these conditions.

Method of Collection of Specimens.—The following is a copy of the directions furnished by Prof. Judson Daland for the instruction of his patients:—

Directions for Collecting Urine.—The urine should be collected in a perfectly clean vessel and four ounces sent to the laboratory. Wide-mouthed four-ounce bottles especially adapted for this purpose should be obtained at the drug stores, and when possible the urine should be directly passed into the bottle.

1. The evening specimen is to be obtained in the following manner. Empty the bladder immediately before the evening meal and discard this urine. From the urine first passed after the evening meal, take four ounces and note the hour when voided.

2. The second specimen is obtained from the urine first passed upon arising in the morning. Note the hour when the urine was passed.

To obtain the total quantity of urine passed in 24 hours. On the day when the observation is begun, at a definite hour, empty the bladder,

and discard this urine. All the urine passed afterward is to be collected in a suitable, clean, dust-proof receptacle and kept in a cool place. The following day at the same hour, when the bladder is first emptied and the urine discarded, again empty the bladder. This urine should be added to complete the total amount for twenty-four hours, which should be expressed in ounces. After the total amount of urine has been collected and thoroughly mixed, send 4 ounces of the mixture.

Example. Observation began on January 1st, at 8 A.M. The bladder is emptied at 8 A.M.; this urine is discarded; the urine passed during the day and night is saved. The next morning, January 2d, at 8 A.M., the bladder is again emptied, and this urine is added to complete the total quantity for twenty-four hours. A label on which is written the name, date, and time when the urine is passed should be *pasted on the bottle.*

The Twenty-four Hours' Specimen.—For accurate results, particularly by quantitative methods, it is necessary to examine a portion of the mixed urine voided during twenty-four hours. Such a specimen should be examined within six or twelve hours after the collection is complete; this will usually prevent errors due to the processes of decomposition and putrefaction which might destroy the formed elements.

Catheterized Specimen.—Catheterization is often resorted to in order to obtain urine free from contamination, which might enter it from the lower part of the urinary tract. For specimens of urine from one or both kidneys the ureters may be catheterized.

Urine kept at room-temperature, particularly in summer time, readily undergoes decomposition and putrefaction. These changes render it unfit for examination.

DECOMPOSITION CHANGES IN NORMAL URINE.

If no method of preservation of the sample is employed, the fresh urine, which is clear of acid reaction and showing no deposit, will gradually undergo the following changes:—

1. The lower part grows cloudy from sedimentation of mucus, cells and other detritus; the urine is still acid.

2. This sediment gradually settles to the bottom and may show minute crystals of uric acid; the urine is less acid.

3. Uniformly cloudy from beginning, precipitation of phosphates. The urine is very faintly acid or neutral.

4. Very turbid from precipitation of phosphates and development of bacteria. Copious sediment of triple and amorphous phosphates, bacteria, ammonium urate, and epithelial *débris.* Alkaline reaction and ammoniacal odor.

PRESERVATION OF SAMPLE.

When, for any reason, it becomes necessary to delay the examination of urine past the time when decomposition changes usually occur, these changes may be retarded in a number of ways: 1. By refrigeration. 2. By the addition of two or three grains of chloral for each ounce of urine. 3. By the addition of ten drops of 4-per-cent. formaldehyde solution for each ounce of urine. 4. By shaking with chloroform in the amount of five drops to the ounce of urine.

DESCRIPTION AND IMPORTANCE OF THE URINE.

The urine is an aqueous solution of the by-products of metabolism, so far as these are not excreted or eliminated by the lungs, bowels, or skin. It is the most important excretory product of the body, and is the medium through which the end-products of nitrogenous metabolism and the soluble mineral salts are almost exclusively excreted under normal conditions. Abnormal products of metabolism and many substances which have found their way into the circulation from without and which are foreign to the body, are likewise carried out in solution.

Not less than 50 per cent. of the total fluid ingested daily is excreted as urine.

General Characteristics.—Normal urine is perfectly transparent when voided, but soon becomes turbid, and on standing deposits a light flocculent sediment composed of a mucinous body and a few epithelial cells and leucocytes. If the urine is kept cold and care is exercised to exclude the entrance of micro-organisms, the upper portion of the urine will remain clear indefinitely. Ammoniacal fermentation, due to the activity of the *bacterium urea* and the *micrococcus urea*, causes cloudy urine from the precipitation of the phosphates.

Bacteria-free urine may, in winter, become cloudy because the contained urates are less soluble in cold urine than in warm urine. On standing the urates settle to the bottom, and the supernatant liquid remains clear as long as bacterial contamination does not occur.

Passage of Turbid Urine.—In man the passage of turbid urine is always abnormal, except during the first days of life,

when the turbidity is due to the profuse desquamation of epithelial cells and the relatively large amount of urates.

PHYSICAL CHARACTERISTICS OF THE URINE.

The Color.—The color of urine normally varies from a light yellow to a dark amber. This is largely influenced by the concentration of the secretion and by the reaction. The pigmentation is due chiefly to the presence of a substance called urochrome (derived from the biliary pigments) and indoxyl potassium sulphate (indican). Acid urine is always darker than alkaline; the color is naturally lighter when the excretion is abundant than when it is scant.

Deviations from the normal color are notably observed in certain diseases and during the administration of certain drugs. It may also occur in apparently healthy individuals in consequence of certain undetermined anomalies of metabolism.

Pale urine may occur as a neurosis or during the course of certain nervous diseases, particularly in epilepsy and hysteria. It may be a symptom of chronic nephritis or of diabetes.

Dark urine, which is clear, occurs in the course of most acute fevers, and is due to the presence of uro-erythrin. A smoky color denotes the presence of decomposed blood, as in acute nephritis. *Blood-red* or *pink urine* usually denotes the presence of fresh blood. Recently Bar and Duaney[1] have called attention to a false bloody urine due to the activities of a pseudomembranous and chromogenic bacterium.

Yellow-brown or *greenish urine* suggests the presence of bile. *Brownish urine* occurs in melanosis and after the ingestion of rhubarb, senna, or tannic acid.

Smoky-brown urine indicates the presence of the end-products of ingested carbolic acid or its analogues.

Pale greenish urine, with a high specific gravity, usually indicates glucose.

White urine denotes the presence of pus or chyle.

Whitish turbidity denotes: 1. Pus. 2. Phosphates. 3. Ammonium urate.

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[1] Le Progrès Medicale, Mar. 24, 1906.

Urine which Darkens on Standing.—Some urine, after standing for a variable period, becomes dark-brown or black. This may be due to the presence in the specimen of melanin or of phenol. Non-pathologic phenol urine or "carbol urine" may arise from medicinal or surgical treatment with phenol or closely allied compounds. Under these conditions the end-products in the urine usually appear as resorcin. Such urine, if alkaline when voided, rapidly darkens on exposure to the air, and if acid becomes dark more slowly, as the alkaline reaction gradually develops.

Under certain pathologic conditions the urine may darken on exposure to the air, owing to the presence of *alkapton*. This condition is known as *alkaptonuria*. A similar darkening in pathologic urine may result from the presence of a coloring matter termed *melanogen*.

Test.—To differentiate between the phenols and melanogen: Add bromine water to the suspected urine. In the presence of phenols there will be produced a permanent, yellow precipitate. A primary yellow precipitate which gradually darkens, becoming finally brown or black, indicates melanogen.

Corroborative Test.—Add to the fresh undarkened urine a few drops of dilute solution of neutral ferric chloride. A violet discoloration denotes phenols, brown or black denotes melanogen.

It must be remembered that urine containing alkapton will give many of the chemical tests for sugar which depend on a reduction process.

The Odor.—Freshly passed normal urine has a peculiar characteristic aromatic odor, resulting from the contained volatile acids. Decomposing urine has the characteristic odor due in part to the free ammonia resulting from the decomposition of urea.

Urine which is ammoniacal when freshly passed, points to a pathologic fermentation occurring in the bladder, usually accompanying cystitis.

A putrid odor denotes putrefactive change occurring in pus or other albuminous substances.

An odor resembling acetone is occasionally observed and denotes diabetes mellitus.

Urine containing cystin may, upon standing, develop the odor of HYDROGEN SULPHIDE.

Some articles of food, as asparagus and onions, and certain aromatic medicines, as turpentine and copaiba, may give characteristic odors.

THE AMOUNT.

The *average daily excretion* of urine in the United States is somewhat less than in foreign beer-drinking countries. A fairly normal average for the United States may be stated to be between 1200 and 1600 cubic centimeters, or about fifty ounces for men, while for women it is slightly less. Generally speaking, the amount will vary in inverse ratio to the insensible perspiration: hot weather diminishes and cold weather increases the amount. Profuse perspiration, sweating, vomiting, and diarrhea all decidedly diminish the amount. Children, and especially nursing infants, on account of the preponderance of liquid in their food, excrete a proportionately larger amount of urine, compared with body-weight, than do adults.

The normal amount of urine passed in twenty-four hours is consequently subject to wide variations, depending on the amount of fluid ingested, the character and the quantity of the food, the process of digestion, the blood-pressure, the surrounding temperature, the emotions, sleep, exercise, age, sex, and body-weight. During repose much less urine is excreted than during activity, hence the excretion during the night is less than during the day. The maximum secretion is usually observed during the first few hours succeeding a hearty meal.

Artificially the excretion may be increased by substances which have a tendency to raise blood-pressure, as tea, coffee, and alcohol. Many drugs bring about the same result. The most important of the medicinal diuretics are digitalis, squill, broom, juniper, nitrous ether, urea, etc. Distilled water also possesses distinct diuretic properties.

The quantity of urine in pathologic conditions depends, first on the condition of the secreting renal parenchyma and, secondly, upon the condition of the blood-current in the kidneys. It will therefore be affected in general by circulatory disturbances, as well as by disease of the kidney itself. To appre-

ciably alter the amount of the excretion, both kidneys must be diseased, for if one kidney be healthy it will assume vicariously the function of the other organ. This compensatory action regularly occurs after extirpation of one kidney.

As a rule the more *acute* the *nephritis* the more the excretion of urine will fall below normal, while the more chronic the process the more will the amount exceed normal. This increase reaches its maximum in the true contracted kidney where the volume excreted in twenty-four hours may be very great. *Diseases of the heart and lungs*, leading to chronic passive congestion, will diminish the total amount of the urine, a condition evidently dependent upon interference in the renal circulation.

It is evident, therefore, that the determination of the total output of the kidneys and an observation of the variation in the amount during the course of disease, may be of great diagnostic and prognostic value.

The increased excretion of urine following convulsions, particularly the hysterical variety and after attacks of angina pectoris, is probably dependent upon some vasomotor disturbance. Many alterations in the volume of the urine depend upon quantitative and qualitative variations in the substances eliminated, and are primarily induced by disturbances in metabolism.

Anuria, or entire failure to void urine, may be due either to complete suppression of the secretion in the kidneys or to obstructions in the urinary tract.

Hydruria.—This is a state of the urine in which the fluid is increased out of proportion to the solids, and is usually associated with an increase in the total twenty-four hours' output.

Polyuria.—This term is applied to an increase in the elimination of the urine as a whole, both fluids and solids.

Oliguria is applied to a diminution in the total excretion of the urine.

Polyuria may be noted in the following conditions: 1. Diabetes mellitus. 2. Diabetes insipidis. 3. Chronic interstitial nephritis. 4. Amyloid disease of the kidney.

Oliguria is met under the following circumstances: 1. Valvular heart-disease. 2. Diminished blood-pressure (see section on blood-pressure). 3. Acute articular rheumatism. 4. Chronic parenchymatous nephritis. 5. Acute congestion and

inflammation of the kidneys. 6. Decreased cell activity in shock. 7. Failure of nutrition preceding death.

SPECIFIC GRAVITY.

The average specific gravity of normal urine of 1500 centimeters (fifty ounces) volume for twenty-four hours, is about 1020. Slight variations (1015 to 1028) from this standard are consistent with perfect health and depend chiefly upon the character of the food ingested, the quantity of water taken, and upon the state of metabolism. Under certain conditions of apparent health, as after a hearty meal, the specific gravity of the individual specimen may be as high as 1035, and after excessive ingestion of fluids it may temporarily fall to 1005.

Under pathologic conditions the specific gravity may vary between 1001 and 1055 or even higher. If the diet consists largely of nitrogenous food it will furnish a relatively larger amount of solids; in consequence the specific gravity of the urine will be increased. Active muscular exertion also tends to raise the specific gravity by increasing tissue catabolism. Copious diaphoresis may bring about a concentration of the urine by diminishing the amount. Fasting has a similar effect.

The specific gravity usually varies inversely with the volume both under normal and pathologic condition. A scanty urine is more concentrated than a profuse one. Diabetes mellitus forms an exception to this rule, since the presence of sugar produces a high specific gravity in spite of the excessive amount of the excretion. This fact is so characteristic that a tentative diagnosis may be made upon this alone.

From the foregoing it is evident that, from a clinical standpoint, it is necessary to consider the specific gravity, the total solids, and the total volume together, as they are intimately and logically related to each other, and that the determination of the specific gravity is of greatest value and significance when the total volume of the urine for twenty-four hours is known. Also that the chief clinical value of the study of the amount and of the specific gravity is to aid in the estimation of the total solids of the urine (see below).

The specific gravity of the urine is usually determined with

the aid of a hydrometer or urinometer, but is more accurately determined by the Westphal balance. Only approximately correct results may be obtained with the urinometer.

The Use of the Urinometer.—The scale of the urinometer is usually marked in regular intervals from 1000 to 1060 (Fig. 39). To insure ease and accuracy in reading, these markings should not be too close together. Many urinometers are inaccurately made, so that before purchasing an instrument it is always well to compare it with a standard instrument, or at least to ascertain that it floats at the 1000 mark in distilled water at the standard temperature (15° C. or 60° F.). Although a

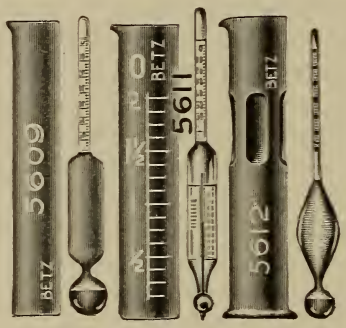


Fig. 39.—Various Forms of Urinometers and Urinometer Cylinders.

large instrument is more accurate, a small one, requiring less urine, is more convenient and must frequently be employed for lack of sufficient urine in which to float the larger instrument.

To determine the specific gravity of the urine: After allowing the urine to cool to room temperature (about 60° F.), it is poured into the urinometer tube (the urinometer and an appropriately sized glass cylinder are usually sold together), and the urinometer immersed in the urine; then with the eye on a level with the surface of the urine, the division of the scale is read off which corresponds to the lowest part of the curve of the meniscus. To insure accuracy the containing cylinder should be sufficiently large to allow the urinometer to float freely and not come in contact with the sides. All bubbles and froth should

be removed from the surface of the fluid by means of filter-paper. If the urine is cooler than 15° C., one-third of a urinometer unit should be subtracted for every degree centigrade below the standard temperature. If warmer an appropriate addition should be made. Since the specific gravity of individual urine specimens vary greatly during twenty-four hours, it is necessary that the specific gravity should be taken from a mixed specimen of the twenty-four hours' collection. A reading taken from any single specimen is of little clinical significance.

If the specimen is too small to work with in the ordinary way, it is a very simple matter to dilute with a known proportion of distilled water. Estimate the specific gravity of the diluted urine and then calculate the specific gravity of the specimen at least approximately.

The Westphal balance is an extremely accurate method of determining the specific gravity, carrying the reading to the fifth figure (fourth decimal). The method of employing the Westphal balance is as follows:—

When the instrument is mounted the glass plummet which is suspended from one end of the beam will balance in distilled water at 15° C. and represents 1. Now pour the urine into the jar until the twist in the platinum wire is below the surface. Weighing is accomplished as follows: Place the first rider upon the end of the beam above the float, then place the second rider in the first notch to the left on the scale on the beam. If the plummet rises, place the rider on the second notch. If now the beam balances and the temperature of the urine is 15° C., the specific gravity of the urine is exactly 1020. If the float still rises, take the third rider and find the notch in which the beam balances or nearly so. If the beam balances with the third rider in the fourth notch, the specific gravity is exactly 1024. Should, however, the float still slightly rise, take the fourth rider and find the exact balance, and if this is in the sixth notch the specific gravity is exactly 10246. In other words, the second rider (in size) gives the third figure, the third rider the fourth (third decimal), and the fourth rider the fifth figure or the fourth decimal. With a little practice determinations with the Westphal balance are rapidly and accurately made. Care should be exercised to see that no air bubbles become attached to the cord

or float, and that temperature corrections are made as directed below.

Corrections for Temperature.—The temperature of the urine immediately after being voided ranges from 85° to 95° F. (29.5° to 35° C.); therefore in taking the specific gravity of fresh urine its temperature must be observed, and for every seven degrees Fahrenheit that the thermometer indicates above the temperature at which the instrument is standardized, one degree should be added to the specific gravity, as indicated by the instrument.

METHOD OF ESTIMATING THE TOTAL SOLIDS.

The *accurate chemical method* of weighing is too difficult and tedious for general clinical work, descriptions of which may be found in works on physiologic chemistry.

The normal average weight of solids is 65 grams. The specific gravity is a pretty accurate index of the amount of solids excreted by the urine when this has been determined from the twenty-four hours' urine.

The solids excreted in 1 liter of urine may be approximated in grains by multiplying the last two figures of the specific gravity by 2.2337 (Vierodt's factor).

Trapp's Formula.—Multiply the last two figures of the specific gravity by two, and the result will represent the parts of solids in 1 liter of urine. Example: If the specific gravity is 1023, then 23 times 2 will equal 46 parts of solids per 1000 cubic centimeters.

Bird's Formula.—The last two figures of the specific gravity about represent the grains of solids in a fluidounce of urine tested. Thus, a specific gravity of 1022 would contain approximately 22 grains of solids per ounce of urine.

Metz's Formula.—Multiply the last two figures of the specific gravity by 0.00233, and multiply this product by the total twenty-four hours' volume in cubic centimeters. The final product will be the total weight of solids expressed in grams. Example: Specific gravity = 1024; then 24 x 0.00233 x 1500 cubic centimeters equals 87.27 grams of solids in twenty-four hours' excretion.

THE REACTION.

The reaction of the twenty-four hours' urine is usually acid, sometimes amphoteric, and rarely alkaline. The normal acidity is not due to the presence of free acids, but to acid salts, chiefly acid phosphates. Primarily the reaction of the urine depends upon the character of the diet. Increase in ingestion of food-stuffs containing alkaline salts (vegetables), or when such salts are formed within the body from organic acids contained in the food (fruits), will tend toward an alkaline reaction when the urine is voided. The elimination of alkaline urine in this instance is due to the presence of a fixed alkaline, and therefore cannot be considered pathologic.

It has already been stated that the acid reaction of urine is normally due chiefly to the acid phosphates. Besides these a certain amount of the neutral phosphates of calcium, ammonium, and of sodium usually occur, and it may happen that the acid and neutral phosphates are present in such proportions that the sample will turn blue litmus red and red litmus blue. Such a urine is said to be *amphoteric*.

Urine, when allowed to remain exposed to the air at room-temperature, gradually undergoes ammoniacal fermentation. This is due chiefly to the action of certain micro-organisms upon urea, which is decomposed by a hydrolytic process into ammonia, carbon dioxid, and water (see page 219).

The result of this development of an alkaline reaction is that the soluble phosphates of the alkaline earths are precipitated as tri-calcium phosphate and ammonio-magnesium phosphate; at the same time the soluble urates are transformed into the insoluble ammonium salt (see Fig. 42).

Test.—In order to determine whether the alkalinity of a given specimen is due to a fixed or to a volatile alkali (ammonia), a strip of red litmus is clamped into the cork of the bottle so that it does not touch the liquid but remains dry. Volatile alkali will gradually turn the paper blue, while a fixed alkali will not affect the paper unless the alkaline fluid touches it.

In specimens where the degree of alkalinity or acidity is so slight that the litmus-paper test leaves the examiner in doubt, the uncertainty may be eliminated by the following expedient:

Take two pieces of litmus-paper, one red and the other blue; allow the urine to come in contact with but half of each strip of paper; then with distilled water allow the whole extent of both strips to become wet. This eliminates any change in the paper due to the presence of moisture alone, and brings out sharply slight changes in the color of the litmus-paper by allowing comparison between the adjacent portions of each strip of paper.

DETERMINATION OF TOTAL ACIDITY OF THE URINE.

Accurate determination of urinary acidity is a difficult and tedious procedure, requiring considerable technical skill. For general clinical purposes Folin's method is practical, and has the advantage of being rapid and simple. It is important to begin with a perfectly fresh sample, in order to exclude change in reaction due to fermentation and decomposition (see page 219). Proteid, if present, must be removed by heat and acetic acid, in which case a known amount of acetic acid must be employed and this taken into consideration in the titration.

FOLIN'S METHOD FOR TOTAL ACIDITY.

Twenty-five cubic centimeters of urine are treated with 15 to 20 grams of powdered potassium oxalate and one or two drops of a 1 per cent. alcoholic solution of phenolphthalein. The mixture is shaken rapidly for one or two minutes and titrated at once with a tenth-normal sodium hydrate solution (for preparation of normal and decinormal solutions see Appendix, page 416.) until a faint, distinct, permanent pink color is obtained. It is advisable to shake the flask during the titration so as to prolong the effects of the potassium oxalate. The acidity is expressed in terms of the amount of tenth-normal sodium hydrate solution necessary for neutralization of the twenty-four-hour amount of urine. This is expressed as T, which is, on an average, 617.

If the urine to be tested is alkaline, then first add to the 100 cubic centimeters exactly 20 cubic centimeters of the one-tenth normal HCl solution. Stir well, and then proceed as in

case of the acid urine, deducting the number of cubic centimeters $\frac{n}{10}$ NaOH from the 20 cubic centimeters $\frac{n}{10}$ HCl used and then calculating as T the total neutralization of the twenty-four-hour specimen.

Increased Acidity.—The urine is frequently found hyperacid in:—

1. Fevers.
2. Inflammations of the liver.
3. Hyperchlorhydria.
4. Acute rheumatism.
5. Lithemia.
6. Neurasthenia.

Alkaline urine may be passed under the following conditions:—

1. Undue retention of urine in the bladder.
2. When there is residual urine.
3. Presence of urea-decomposing bacteria in the bladder.
4. Chlorosis.
5. Organic nervous diseases.
6. In marked degrees of general debility.

CHEMICAL COMPOSITION OF THE URINE.

A general idea of the chemical composition of the urine and of the quantitative variation of the individual components may be had from the accompanying table (after Simon). The individuals from whom the urine was obtained were adults. Their habits, diet, etc., may be taken to be that of the average American city dweller.

It must be borne in mind that tables of chemical composition are based upon averages taken from a large number of complete analyses, the composition of which, even in perfect health, covers a greater range of variation than indicated in the following table:—

CHEMICAL ANALYSIS OF THE URINE (Simon).

	Grams.
Water	1200 to 1700
Solids	60
Inorganic solids	25.0 to 26.0
Sulphuric acid (H_2SO_4)	2.0 to 2.5
Phosphoric acid (P_2O_5)	2.5 to 3.5
Chlorine (NaCl)	10.0 to 15.0
Potassium (K_2O)	3.3
Calcium (CaO)	0.2 to 0.4
Magnesium (MgO)	0.5
Ammonia (NH_3)	0.7
Fluorids, nitrates, etc.	0.2
Organic solids	20.0 to 35.0
Urea	20.0 to 30.0
Uric acid	0.2 to 1.0
Xanthin bases	1.0
Creatinin	0.05 to 0.06
Oxalic acid	0.05
Conjugate sulphates	0.12 to 0.25
Hippuric acid	0.65 to 0.7
Volatile fatty acids	0.05
Other organic solids	2.5

THE INORGANIC CONSTITUENTS OF THE URINE.

The inorganic constituents of the urine represent the excess of mineral salts that find their way into the blood from the digestive tract or which develop within the body during the processes of metabolism, especially during albumin decomposition. We therefore find that exercise and the ingestion of large amounts of food, as well as the increased cell activity occurring in acute fevers, lead to an increased elimination of salts, and conversely smaller amounts of salts are eliminated when the intake of food in general is restricted. These statements apply particularly to the phosphates.

The bases which are found in the urine in combination with hydrochloric acid, phosphoric acid, and sulphuric acid, are chiefly sodium, potassium, calcium, magnesium, and ammonium. It is believed that the mono-acid phosphates of the alkaline earths are held in solution by sodium chlorid, and also by the di-acid sodium phosphates, to which latter the acidity of the urine is largely due.

While the greater portion of the sulphuric which results

from albumin decomposition is found in the urine combined with inorganic bases, a variable fraction also occurs united with certain aromatic substances which are developed in the intestines during putrefaction and decomposition. The resulting bodies are spoken of as the ethereal or conjugate sulphates. They comprise the alkaline salts of indol, skatol, and phenol.

The Phosphates.—The amount of phosphoric acid excreted by the healthy individual in twenty-four hours ranges from 2.3 to 3.5 grams, the average being 2.8. In the urine the phosphoric acid is found in part combined with the alkaline earths—earthy phosphates—and in part with the alkalies as the alkaline phosphates. The alkaline bases represent about two-thirds of the total phosphoric acid in combination. The earthy phosphates are insoluble in water, but are soluble in dilute acids. They comprise the phosphates of calcium and magnesium. The calcium phosphates predominate. In acid urine the earthy phosphates are in solution, while in alkaline urine they are precipitated. They are thrown down by heat, and also when the reaction of the urine becomes alkaline, whether from a course of internal alkaline medication or from the putrefaction of the urea of the urine. If, during decomposition of the urine, the contained acid phosphates are acted upon by the ammonia resulting from urea decomposition, ammonium-magnesium phosphate (triple phosphate) is formed and appears in the urine as the characteristic prismatic or coffin-lid crystals.

The alkaline phosphates comprise the phosphates of sodium and potassium; of these the sodium is the more abundant. These, unlike the earthy phosphates, are easily soluble in water and in alkaline fluids. The alkaline phosphates form the chief bulk of urinary phosphates. The normal urinary acidity depends upon the presence of these acid phosphates, and not upon the presence of free acid.

While the bulk of the phosphates in the urine is derived from the decomposition of food-stuffs, a part also is derived from the breaking down of the highly complex organic bodies: lecithin and nuclein.

Clinically, the execretion of phosphoric acid and its determination is of very little significance, since it is so largely dependent upon the influence of diet, exercise, etc.

Detection.—If neutral or alkaline urine is heated in a test-tube, a precipitate will be formed which will be found to consist of earthy phosphates. Such a precipitate might be caused by the presence of albumin. To remove the doubt add a few drops of dilute (10 per cent.) acetic acid, when a precipitate due the phosphates will immediately disappear, while if albuminous the cloud will remain or may be increased.

Test for Earthy Phosphates.—To a few cubic centimeters of urine in a test-tube add a few drops of liquor potassii and boil. The earthy phosphates will be thrown out of solution, and may, after settling, be collected upon a filter. Now, to the filtrate add one-third of its volume of "magnesia mixture" (for formula see Appendix). The precipitate thus formed represents the phosphoric acid that was in combination with the alkaline bases, combined now in the form of ammonium-magnesium phosphate.

Estimation of the Phosphates by the Centrifuge.—In a graduated percentage centrifuge tube (see Fig. 51) mix 10 cubic centimeters of urine with 5 cubic centimeters of "magnesia mixture"; invert several times to thoroughly mingle. Revolve in the centrifuge for three minutes. Read off every one-tenth cubic centimeter of precipitate as 1 per cent. by bulk of total phosphates. The average normal percentage by this method is eight. Roughly, each one-tenth cubic centimeter of sediment is equal to about 0.0225 per cent. by weight of $P_2 O_5$.

Determination of the Total Phosphoric Acid.—For this determination the following solutions are required:—

1. A standard solution of uranium nitrate: 20.3 grams of uranium nitrate are dissolved in 1000 cubic centimeters of distilled water, then each cubic centimeter of this mixture is equivalent to 5 milligrams of phosphoric acid.

2. Sodium acetate solution: 100 grams of sodium acetate are dissolved in 900 cubic centimeters of distilled water, and to this 100 cubic centimeters of acetic acid are added.

3. Saturated solution of potassium ferrocyanide.

Method.—Fifty cubic centimeters of urine are placed in a beaker and 5 cubic centimeters of the sodium acetate solution added. The mixture is warmed over a water-bath and uranium

nitrate solution added from a burette as long as a precipitate is formed. If the formation of precipitate is not easily recognized, a drop of the potassium ferrocyanide solution may be added; then, as long as a brown color does not appear where the drop falls, the uranium nitrate solution should continue to be added. The end point in the precipitation reaction is reached when a reddish-brown discoloration appears upon the addition of a drop of the uranium nitrate. The quantity of uranium nitrate solution employed to accomplish complete precipitation is now read off from the scale on the burette, each cubic centimeter of which will equal 5 milligrams of phosphoric acid. This number and the 50 cubic centimeters of urine employed furnished the working bases for calculating the percentage.

The presence of sugar or albumin does not interfere with this reaction.

Separate Estimation of the Earthy and Alkaline Phosphates.—Two hundred cubic centimeters of urine in a beaker are rendered alkaline by the addition of ammonium hydroxid, and set aside for a few hours. The earthy phosphates are thus precipitated and may be collected upon a filter-paper, and after washing with dilute ammonia (1:3) are transferred to a beaker, where they are dissolved with as little acetic acid as possible. Distilled water is then added so as to make the total volume approximately 50 cubic centimeters, when the solution is boiled and then titrated as above. In a second portion of urine the total phosphates are determined as outlined above. Then the difference between the two results will represent the quantity of phosphoric acid present in combination with the alkalies.

Significance.—As has been shown above, the phosphates are dependent upon many uncertain factors which are determined with difficulty and are of little clinical significance. When in a given case the phosphates are constantly thrown out of solution, it may be taken as an indication that the formation of gravel or calculus is impending. If the usual signs of ammoniacal fermentation are present, the significance is plain.

THE SULPHATES.

General Considerations.—The major portion of the sulphates appearing in the urine are derived from the food, and comprise the simple mineral sulphates of sodium and potassium. Only a small portion exists in organic combination as the ethereal or conjugate sulphates.

The three predominating conjugate sulphates are: Phenol potassium sulphate, indoxyl potassium sulphate (indican), and skatoxyl potassium sulphate.

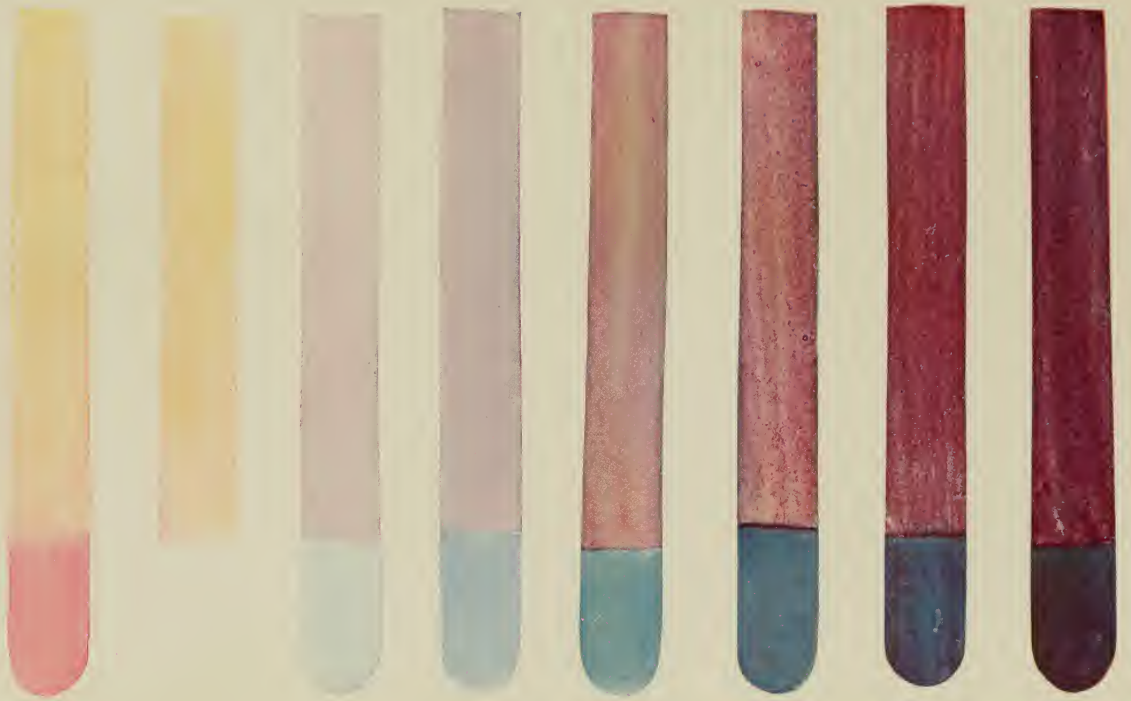
The mineral sulphates comprise about nine-tenths of the total sulphates in the urine.

Test for Mineral or Preformed Sulphates.—Add a few drops of acetic acid (to prevent the formation of barium phosphate) to a test-tube half full of urine. Now, upon the addition of a solution of barium chlorid a white precipitate of insoluble barium sulphate will be formed. This precipitate varies in density from a faint white cloud to a bulk that gives a thick creamy consistence to the whole mixture. One can roughly determine by the amount of precipitate as compared with the known normal standard, whether the sulphates are increased or not.

Test for Conjugate or Ethereal Sulphates.—Mix equal quantities of alkaline barium chlorid (see Appendix) and urine in a test-tube. After allowing a few minutes for the precipitate to form the mixture is filtered. This process precipitates both phosphates and preformed sulphates. The filtrate is now acidified with 5 cubic centimeters of a (one-fifth vol.) HCl solution and then boiled for some time. The presence of ethereal sulphates is indicated by a reddish discoloration of the fluid which in addition becomes turbid.

Potassium Indoxyl Sulphate or Indican.—As this substance represents the characteristic ethereal sulphate, it may be taken as an indicator for the whole group, and the tests for this substance used for estimating the relative amount of the whole group of ethereal sulphates in the urine. The several tests for indican are based upon the fact that an excess of HCl will liberate the indoxyl, which can then, by the addition of an oxidizing agent, be converted into indigo-blue, and finally this can be recognized in small amounts by extraction from the bulk of urine with chloroform.

PLATE VI



Iodine Reaction

Indican Absent

Very Faint Trace

Trace

Large Trace

Moderate Amount

Large Amount

Very Large Amount

Test for Indican (modified Jaffé).—Take 20 cubic centimeters of filtered urine in a test-tube and add 3 or 4 cubic centimeters of chloroform and one drop of a 1 per cent. solution of potassium chlorate, and finally 20 cubic centimeters of HCl. This mixture is to be thoroughly agitated and mixed by pouring repeatedly from one test-tube to another. This should be repeated at intervals of two or three minutes, covering a period of ten minutes. The presence of indican is indicated by a blue discoloration of the chloroform. In the presence of a large amount of indican the chloroform will appear almost black, while the whole mixture will assume a dusky bluish-red color. (For comparative color-scale see Plate VI.)

While a trace of indican cannot be considered pathologic, there are, nevertheless, many specimens, possibly one-third, which fail to show any discoloration of the chloroform.

Caution.—1. An excess of oxidizing agent, either in volume or strength, will prevent a positive reaction through over-oxidation of the indoxyl compound. 2. An excess of chloroform will result in too great dilution of the indigo, causing failure to detect traces of indican. 3. A reddish discoloration of the chloroform is not due to indican. Such a reaction occurs in the urine of patients who are taking iodid, and possibly bromid. The simultaneous occurrence of a red and blue reaction will produce a color bordering on purple. This possible source of error may be removed by the addition of a few drops of a 10 per cent. solution of sodium thiosulphite, which will bleach the pink color due to iodin.

Obermayer's Test.—This test is also a modification of Jaffé's test, with certain improvements suggested by Rosenbloom.[2]

To nearly a half test-tube full of urine add an equal quantity of hydrochloric acid containing 4 grams of ferric chlorid to the liter. Mix by pouring from one tube to another and allow to stand ten minutes. Add about 3 cubic centimeters of chloroform and again mix by pouring from one tube to another, then permit it to stand fifteen to thirty minutes. The supernatant fluid above the chloroform is now poured off and the test-tube filled with water. This accentuates the blue color due to the

[2] N. Y. Med. Jour., October 25, 1913.

presence of chloroform. Thymol, if added to the urine as a preservative, affects the end action, the thymol giving the urine a violet color.

Should protein matter be present in the urine, it must be removed by boiling, precipitation with dilute acetic acid, and filtering. The filtrate is used in testing for the indican.

APPROXIMATE QUANTITATIVE DETERMINATION OF INDICAN.

F. C. Askenstedt[3] recommends the following modification of the Jaffé technic for the determination of indican.

The following solutions are used:—

A. 0.4 per cent. solution of iron perchlorid in HCl.

B. A true solution of indigo blue in sulphuric acid. This strength is of such a solution that one drop holds exactly 0.000165 milligram of indigo blue. (This solution keeps well in amber-colored bottles.)

C. An appropriate 1 : 5000 solution of picric acid in alcohol.

D. Denatured alcohol.

The test is carried out as follows: To 10 cubic centimeters of urine in a test-tube add 10 cubic centimeters of the ferric chlorid solution and mix by inverting the tube once; then add quickly 8 cubic centimeters of chloroform, and extract the indigo in formation by shaking the tube 400 times, holding it in a horizontal position. After this let the chloroform fall to the bottom of the tube, then pour off most of the supernatant fluid, fill the tube nearly full with water, invert it a few times to wash the chloroform and let this again precipitate in the tube, and pour off most of the water. Repeat twice this process of washing, taking care that no chloroform escape with the wash-water, and allowing not more than 2 or 3 cubic centimeters of the last wash-water to remain over the chloroform. Now add from 13 to 15 cubic centimeters of alcohol and mix by shaking. A clear blue fluid should result. If hazy, add one or two cubic centimeters more of alcohol until the fluid clears up. Compare the color of this fluid with an equal quantity of a standard solution of indigo blue in the second test-tube by holding the two test-tubes in front

[3] N. Y. Med. Jour., October 9, 1909.

of a white surface. This standard solution is made by pouring into the empty second tube a quantity of water equal to the amount of the fluid in the first tube, and then dropping the stock solution of indigo blue into the water, inverting the tube after each drop, until both solutions have the same amount of blue color. If this requires four drops of the stock solution the percentage is 0.0004; if five drops, 0.0005; if six drops, 0.0006, etc.

Suggestions.—Several drops of the picric acid solution may be added to the standard solution in the test-tube in order to balance an occasional greenish tinge appearing in the indican extract.

Advantages.—Neither albumin nor bile interferes with this estimation. Sugar reduces it. To compensate for indican lost during the technic, add 20 per cent. of the amount determined for the final result. Urine containing more than 0.002 indican, or which has a blackish extract, should be diluted with equal parts of water and retested. The presence of formaldehyde gas in the laboratory may prevent indigo formation.

SKATOL.

Skatol Potassium Sulphate or Skatol.—A certain amount of skatol sulphate is found in the urine in conjunction with the similar indoxyl compound, indican. This substance will produce a red discoloration of the chloroform with the Jaffé test which is not removed by a 10 per cent. solution of sodium thiosulphate, as is the pinkish discoloration due to iodin and iodids. This substance is also known as indigo red, skatoxyl red, and urorubin. It has the same significance as indican.

Rosenbach's Test.—Boil a few cubic centimeters of urine, and then add drop by drop nitric acid and continue boiling. In the presence of skatol a deep-red color appears and the foam produced by shaking is a bluish red.

Amylic Alcohol Test.—Proceed as in the Jaffé test, finally overlaying the whole with pure amylic alcohol. Gentle agitation will extract the skatol, which will produce a dirty brown or black discoloration of the amylic alcohol as it floats on the top of the tube.

THE CHLORIDS.

The quantity of chlorids in the urine is usually decreased in: 1. Most febrile diseases. 2. Nephritis. 3. Wasting diseases. 4. Pneumonia.

Approximate Estimation of the Chlorids.—To a few cubic centimeters of urine in a test-tube, add a few drops of nitric acid, boil and filter to remove the albumin. Add to the filtrate a few drops of a 10 per cent. solution of silver nitrate. The abundance of the white cloud will roughly indicate the amount of chlorids present.

Accurate Methods of Quantitative Determination.—Method of Salkowski-Volhard. Take 10 cubic centimeters of urine in a beaker and dilute with 50 cubic centimeters of distilled water, and then treat with 4 cubic centimeters of concentrated nitric acid and 15 cubic centimeters of a standard silver nitrate solution. The mixture is then further diluted with distilled water up to 100 cubic centimeters, and after thorough agitation is passed through a dry filter. In a carefully measured portion of the filtrate the excess of silver is carefully titrated with a solution of potassium sulphocyanid of such strength that 25 cubic centimeters correspond to 10 cubic centimeters of the standard silver solution. A few drops of a saturated solution of ammonio-ferric alum serve as an indicator. The amount of silver solution employed to precipitate the chlorids in the 10 cubic centimeters of urine is then calculated. The number of cubic centimeters necessary for this precipitation is multiplied by 0.01, which will indicate the amount of chlorids in 10 cubic centimeters of the urine calculated as sodium chlorid. The presence of albumins or of sugar does not interfere with this reaction. (For test solutions see Appendix.)

PURDY'S CENTRIFUGAL METHOD OF ESTIMATING CHLORIDS.

This method, while having nothing in common with the accuracy of the preceding one, is very convenient and has the advantage of yielding quick clinical results.

Ten cubic centimeters of clear, filtered, albumin-free urine are placed in a centrifuge tube, which is graduated to 15 cubic

centimeters. One cubic centimeter of strong nitric acid and 4 cubic centimeters of a 5 per cent. solution of silver nitrate are then added. The tube is shaken by inversion and the mixture allowed to stand for a few minutes, after which it is placed in a centrifuge and whirled for three minutes at the rate of 1200 revolutions per minute. The bulk percentage of silver chlorid is then read off, from which the percentage by weight, both of sodium chlorid and of chlorin, equivalent to the precipitated silver chlorid may be calculated. One per cent. by bulk represents 0.13 per cent. by weight of NaCl and 0.08 per cent. of chlorin.

As previously stated, the amount of chlorin in the urine depends upon the amount ingested, ranging normally between 10 and 15 grams in twenty-four hours.

THE ORGANIC CONSTITUENTS OF THE URINE.

GENERAL CONSIDERATIONS.

The organic constituents of the urine comprise the normal end-products of nitrogenous metabolism within the body, also various products of albuminous putrefaction which have found their way into the general circulation from the intestinal tract. Finally constant pigments which bear a relation to the normal blood-pigments and various substances of obscure origin are encountered. Under abnormal conditions we may meet with normal constituents of the blood which do not ordinarily appear in the urine, while in pathologic conditions we meet various products of abnormal metabolism. Just as the composition of the average dietary varies in different localities, and is modified from time to time by design or by seasons, so will the urine show material variations in its normal end-products of nitrogenous metabolism. Much clinical work has been done in an effort to determine the significance of many of these substances, but up to now little of definite value is known of most of them; so only those findings which are of actual clinical value will be considered on the pages following.

UREA.

Urea was first synthetically prepared from ammonium cyanate in 1828 by Wohler. Formerly it was supposed that urea resulted from uric acid through a process of oxidation, and that this was its only source. Now this error is well known, and while it is recognized that the formation of urea from uric acid is possible and that a small portion of the total amount may be derived in this manner, modern research has shown that in man urea is largely derived from the destruction of the nucleins within the body, and that the sources of the nucleins are both the tissue cells and the cells of animal foods ingested.

It has been repeatedly shown that during the decomposition of albumins by means of acids and alkalies, as during the process of tryptic digestion and albuminous decomposition, a large amount of mono-amido-acids results. And it is supposed that these bodies probably represent the intermediary products in the transformation of albuminous nitrogen into urea. Under certain pathologic conditions these acids may appear in the urine, and when they are there noted the elimination of urea is much diminished. In health, however, this does not occur, and on examination of the different tissues of the body such acids are found only in traces. We must conclude, therefore, that these acids, supposing them to occur as the primary products of albuminous decomposition within the body, are transformed at once into other substances, which in turn give rise to urea.

It has also been shown that the amido acids yield carbamic acid on oxidation, and that on alternate oxidation and reduction urea can be produced from the ammonium salt (ammonium carbamide). While it is generally assumed that urea is largely referable to a transformation of the mono-amido-acids into ammonium carbamate, and while it has also been shown that such a transformation does actually occur, it must be remembered that at best only traces of these amido acids are found in the tissues.

From these conclusions it is reasonable to believe that the greater portion of albuminous nitrogen is set free from the various organs of the body in the form of an ammonium salt of paralactic acid, and that this salt is now generally conceded to be the antecedent of urea.

It is probable that a certain amount of urea is produced in the body in a number of ways, and there is ground for the belief that its formation is not confined to a single organ. The greater part is, without doubt, produced in the liver. In corroboration of this fact it has been repeatedly shown that in disease of this organ, when accompanied by extensive destruction of the glandular elements, a diminished amount of urea is found in the urine, while ammonia and lactic acid are found in increased amounts. In certain cases of this class as much as 37 per cent. of the total urinary nitrogen has been found in the urine in the form of ammonia.

If we accept the modern doctrine that urea originates not only in different ways, but that it may also be formed in other organs beside the liver, then we can understand why it is that in certain diseases of the liver the diminution in the amount of urea excretion is not always proportionate to the extent of parenchymatous degeneration, and that no case has yet been reported in which the excretion of urea has ceased altogether.

Nitrogenous Equilibrium.—The albumins are the ultimate source of the urea, and according to Pettenkofer they exist within the body in two forms, viz.: as organized albumin, which is built up into tissues, and the so-called circulating albumin, which is present in excess of the actual requirements of the body and which may be broken down directly and eliminated in the urine without ever having entered into the construction of the body-proper. This latter portion of the body albumin is said to furnish the bulk of the urea, while the fixed tissue albumin represents the minor but more uniform source. The actual amount eliminated will, therefore, be primarily dependent upon the quantity ingested.

Experiment has shown that under normal conditions of average diet the total urinary nitrogen is practically equivalent to the quantity ingested, barring a small fraction, which escapes digestion and appears in the feces. Such a condition is spoken of as the nitrogenous equilibrium of the body. Of this relation infinite variations exist, which may even vary from time to time in the same individual. If the amount of nitrogenous food is suddenly diminished the amount of urinary nitrogen will also decrease; then if the reduced ingestion remains constant the

nitrogen output, while lowered, will at the same time tend to remain level. If, on the other hand, the nitrogen intake is increased, an increased nitrogen elimination speedily follows, but here a certain fraction will be retained in the body and gradually a higher level of equilibrium will be established.

There are natural limits to this power of accommodation of the system to nitrogenous ingestion and elimination, so that we may find a point which varies in different individuals where a further nitrogen ingestion does not lead to a higher level of nitrogenous equilibrium, and when, consequently, a further retention of nitrogen does not occur. Overfeeding then results in various digestive disturbances. Diarrhea and vomiting may occur through nature's effort to protect the body from a further increase in circulating nitrogen.

Underfeeding, on the other hand, generally leads to increased destruction of the organized albumins. Although for a while the body's store of fats and carbohydrates is capable of protecting the body against an undue loss of nitrogen in this direction, still if the reduced intake of nitrogen continues sufficiently long, death finally ensues.

From the fact that the level of nitrogenous equilibrium varies in different individuals and in the same individual from time to time, it follows that the amount of urea excreted must also vary according to the same irregular manner. Any figures, therefore, which are supposed to indicate the amount of urea eliminated, can be of little and uncertain value unless the actual state of the individual's health is known, also his body-weight, habits as to exercise, the amount of nitrogenous food ingested, etc. Only when we are in possession of an accurate knowledge of these several factors can we say whether the urea excretion is or is not normal.

Certain figures have been compiled by physiologists to indicate the amount of nitrogen which should enter into the composition of the diet and from which we may approximately calculate the amount of urea that should be excreted. By estimating this, or still better, by determining the total nitrogen elimination, we can then determine whether or not the individual is consuming the proper amount of nitrogenous food in his dietary. While such figures may serve as a general guide,

they have mostly been constructed without due regard for the factors above indicated, and should not, therefore, be too implicitly relied upon.

According to Simon, among the well-to-do classes the elimination of from twenty to twenty-five grams of nitrogen in twenty-four hours is about normal, taking the average body-weight of the individual into consideration. A smaller amount is not infrequently met in persons of sedentary habits who may appear to be in perfect health.

Urea in Disease.—While extensive variations occur in the urea excretion of health, still greater variations from the average standard are noted in disease. But here also should be taken into account the amount of nitrogen ingested in relation to the body-weight.

An increased elimination of urea referable to the destruction of organized albumins is frequently observed, but this may in cases be obscured by a deficient nitrogen ingestion unless the total intake of the latter is not definitely known. It is of interest here to note that in certain diseases of the liver in which there is great destruction in the parenchyma, the amount of urea may be markedly diminished, even when a fairly abundant supply of nitrogen is taken in. This will be found due largely to the interference in urea synthesis, and as a secondary result we find that in these cases a considerable portion of the nitrogen appears in the urine in the form of ammonium salts of paralactic acid (see page 242) and of carbamic acid; in extreme cases as mono-amido-acids and as leucin and tyrosin.

Properties of Urea.—Urea crystallizes in colorless, quadrilateral, or six-sided prisms with oblique ends, or when rapidly crystallized in delicate white needles which melt at 132° C., but which are probably decomposed at a temperature of 100° C. They contain no water of crystallization, and are permanent in the air, and easily soluble in cold water, in which they form a neutral solution. With nitric acid, urea unites to form urea nitrate, which crystallizes out in octahedral lozenge-shaped or hexagonal platelets. Urea nitrate is readily soluble in distilled water, but is soluble less readily in water acidified with nitric acid. Its formation is frequently observed when urine is examined cold, with nitric acid, for the presence of albumin. On

heating, the crystals are decomposed without leaving any residue.

Detection of Urea.—1. To detect urea place a drop or two of the suspected fluid upon a glass slide, and after adding a drop or two of nitric acid, warm gently. If urea is present, after partial evaporation and cooling, the microscope will show the characteristic crystals of urea nitrate. These are either rhombic or hexagonal plates, frequently overlapping like shingles on a roof. Their acute angles measure 82°.

2. Add to the suspected fluid, in a test-tube, a few drops of fresh sodium hypobromite. A rapid evolution of gas will indicate the presence of urea.

Since clinical observations are concerned in the total output of urea, it becomes necessary to estimate the quantity or percentage from a sample of twenty-four hours' urine.

Under normal conditions of average health the percentage of urea is two.

The average daily excretion of urea is forty grams or five hundred grains, or about half the weight of the total solids.

Quantitative Estimation of Urea.—This quantitative estimation is determined by calculation from the observed volume of nitrogen gas evolved from a measured quantity of urine by a process of decomposition. For practical purposes one gram of urea is estimated to furnish 37 cubic centimeters of nitrogen gas. The decomposition of the urea contained in the measured volume of urine is accomplished by means of an alkaline solution of sodium hypobromite (see Appendix for formula of Knop's solution). The test is best conducted in a Doremus-Hinds ureameter (see Fig. 40).

The Test.—First pass some urine through the small tube to wet the stop-cock, then fill the large tube with Knop's solution, and the smaller side tube with urine exactly up to the 0 cubic centimeter mark. Now, drop by drop, allow exactly 1 cubic centimeter of urine to pass into the reagent in the large tube. When the bubbles of gas (nitrogen) cease to rise, the fraction of a gram of urea may be read directly from the graduated scale on the larger tube.

Example.—Suppose that after admitting exactly 1 cubic centimeter of urine the level of the fluid stands at the 0.018

mark. Then the 1 cubic centimeter of the sample contained 0.018 gram of urea. To obtain the total amount of urea excreted in twenty-four hours, it is only necessary to multiply this figure by the number of cubic centimeters in twenty-four hours' urine.

URIC ACID.

General Considerations.—Uric acid, like urea, is nitrogenous. The normal proportion of uric acid to urea is as 1 : 45. In health it exists in solution as sodium and as potassium urate. A healthy adult excretes 0.2 to 1.0 gram of uric acid in the course of twenty-four hours. The amount increases physiologically

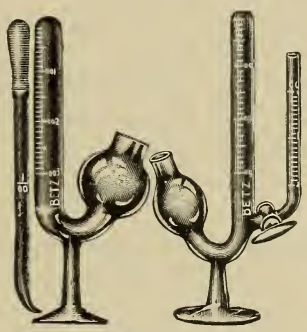


Fig. 40.—Various Forms of Ureometers.

with increased ingestion of food, and pathologically with increased nitrogen metabolism in about the same proportions as urea. The amount of uric acid in urine varies directly with the specific gravity, so that the last two figures of the specific gravity (calculated to four places), multiplied by two, give approximately the number of centigrams of uric acid in the litre. The daily excretion of uric acid is increased in fevers and in leukemia. The relation of the elimination of uric acid to attacks of gout and the so-called uric acid diathesis, is still an unsettled question. Of necessity the elimination of uric acid is increased by the ingestion of uric acid and other purin bodies, as well as by foods rich in nuclein (rich in cells).

Properties of Uric Acid.—Uric acid is practically insoluble in cold water, requiring 18,000 parts of water to dissolve 1 part

of uric acid. It is freely soluble in alkalies and in solutions of the carbonates. Pure uric acid crystals are colorless, transparent platelets, the angles of which are frequently irregular and rounded off. Such crystals are occasionally seen in freshly voided urines, but more commonly they are found in the form of brownish-yellow, whetstone-shaped crystals, occurring singly or in groups (see Plate VIII, *a* and *b*). Uric acid once deposited remains undissolved in acid urine. Owing to the inclusion of the urinary pigments in the crystals, these, when present in appreciable quantity, are spoken of as "brick-dust."

Microscopic Appearance.—The reddish specks observed by the naked eye are found, upon microscopic examination, to be various modifications of rhombic prisms (see Plate VIII, *c, d, e* and *f*). The simpler forms have some resemblance to the conventional lozenge or whetstone.

Significance.—If these crystals deposit in appreciable quantities soon after micturition, it may be considered as a sign of impending gout or gravel formation. Such a deposit, however, is not necessarily evidence that the elimination of uric acid is excessive.

Isolation of Uric Acid.—To 200 cubic centimeters of urine add 10 cubic centimeters of HCl, and let stand for twenty-four hours; the uric acid will then have settled to the bottom of the container, from which it may be collected by decanting, filtering, and finally washing in cold water.

Qualitative Tests for Uric Acid.—Murexid Test: Put the solution supposed to contain uric acid or urates in a porcelain dish, add a drop of nitric acid and evaporate to dryness. After cooling, allow a drop or two of ammonia water to come in contact with the residue. The presence of uric acid or urates will be shown by bright blue or violet (murexid) color.

Schiff's Test.—Having a residue prepared as above, or crystals supposed to be uric acid, dissolve in a test-tube with the aid of a solution of sodium carbonate. Moisten some filter-paper with a 10-per-cent. solution of silver nitrate. Into the center of this paper allow a few drops of the uric acid sodium carbonate solution to fall. In the presence of uric acid the silver nitrate will be reduced to black metallic silver.

Approximate Quantitative Determination. — According to

Gubler, the amount of uric acid in urine may be approximately determined by stratifying the specimen of urine to be tested upon a layer of nitric acid contained in a test-tube, so that the volume of urine to the volume of nitric acid is as 3 : 2. After a short interval uric acid crystals will separate out as a cloudy white ring at the line of junction of the two fluids. If the amount of uric acid in the specimen is increased, the precipitation will be plain in five minutes or less. If diminished it will not appear until later. This determination is of value only when the daily excretion of urine is approximately normal, and if it is diminished in amount it should be diluted with water up to the average amount before applying the test. Obviously the conclusions derived from such a method should be given very slight weight clinically, and then only when the estimation is made with a part of the twenty-four hours' collection, properly diluted as mentioned above. Albumin must first be removed by slight acidulation with dilute acetic acid, boiling and filtering, after which the test should be applied when the urine has cooled to room-temperature.

QUANTITATIVE CLINICAL DETERMINATION OF URIC ACID.

Ruhemann's Test.[4]—This method is a very convenient clinical one, although its results are by no means as accurate as other more complicated methods. What the general practitioner desires, as a rule, is to know whether the uric acid is increased or diminished, not the absolute quantitative values. This method determines the total purin content and is sufficiently accurate for clinical purposes.

It consists in the use of a specially graduated tube, the uricometer (see Fig. 41), in which are placed the reagents and the urine to be tested. The calibrations etched on the tube are devised to give directly the amount of uric acid in parts per 1000. The method depends on the decolorization of an iodin solution by the uric acid of the urine, and the measurement of the amount of urine which must be added to a definite amount of iodin solution to effect this decolorization.

[4] Webster's "Diagnostic Methods," 1912.

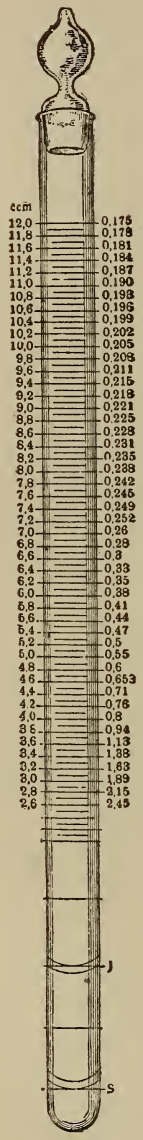


FIG. 41.—RUHEMANN'S URICOMETER.

TECHNIC.—Sufficient carbon disulphid is placed in the tube to bring the lower meniscus of this reagent to the S mark. A solution of iodin in potassium iodid is then added, so that the upper portion of the meniscus coincides with the mark J. (For solution see Appendix, page 404).

The urine is added slowly by means of a pipette until the lowest calibration is reached. The glass stopper is inserted and the contents of the tube mixed by repeated inversion for about fifteen seconds. The carbon disulphid absorbs the iodin, taking on a distinct purple coloration. If this amount of urine does not completely decolorize the iodin, shown by porcelain-like color of the carbon disulphid solution, more urine is added and the tube again inverted for fifteen seconds. This process is continued until repeated shaking of the tube causes the carbon disulphid to assume a pale-pink color. This is the end-point of the reaction, as more shaking of the contents will cause the indicator to assume the characteristic porcelain-white appearance. The time consumed by this test is from five to sixteen minutes. The amount of uric acid is then read off directly from the tube in parts per liter.

CAUTIONS.—Should the urine contain less uric acid than can be read off from the calibrations, a second test is made, adding the iodin solution to the mark midway between S and J, the amount indicated on the tube being, of course, divided by 2. Conversely, should the urine contain more uric acid than is represented by the lower calibration, one adds the iodin solution to the point above J and multiplies his reading by 1.5 or adds the iodin solution to the second mark above J and multiplies the reading by 2.

With this method the urine must be acid in reaction. If the urine contains a sediment of urates, it should be thoroughly shaken before be-

ing added, so that the urates may be in suspension. Any free uric acid which may have separated in the sediment is not determined in this method. Strongly colored urines have no influence upon the decolorization. The presence of sugar does not interfere with the results, but if albumin be present in large amounts it should be removed by acidifying with dilute acetic acid, boiling, and filtering.

THE PURIN BASES IN THE URINE.

These comprise xanthin, hypoxanthin, heteroxanthin, paraxanthin, guanin, and adenin. These bases are normally present in the urine and comprise approximately one-tenth as much as the normal uric acid. The amount of purin bases normally found in the urine in twenty-four hours varies between 0.028 and 0.058 gram. There is at present no practical clinical method of estimating them that is within the scope of the average laboratory equipment.

THE URATES.

General Consideration.—When the urine cools the urates may settle to the bottom of the container as a cloudy reddish-yellow precipitate. This is most likely to occur when the urine is scanty, concentrated, and highly acid. This condition often obtains in fevers, and in congestion or inflammation of the kidneys. The sediment then presents a fairly characteristic appearance, being clay-colored, reddish yellow, or rose-red. It may adhere to the walls of the vessel as a fine, reddish coating.

The ordinary uratic sediment consists of a mixture of the urates of sodium, potassium, calcium, magnesium, and ammonium. Sodium urate predominates. With the exception of ammonium urate, these urates only appear in acid urine. Uratic sediments often contain a few crystals of uric acid which have been formed during the double rearrangement of molecules which resulted in the precipitation of the urates. If urine containing a uratic sediment is decomposed by ammoniacal fermentation, the sediment will be changed to acid-ammonium urate, and this latter is the only urate sediment which occurs in alkaline urine.

If freshly voided urine is kept in a cool place, the precipitation of urates will occur rapidly. This same precipitation occurs at a higher temperature if the amount of urates is in excess of normal or if the urinary acidity is decreased. This precipitate is readily distinguished from phosphates by its prompt disappearance upon the application of heat.

Qualitative Tests.—1. Half fill a test-tube with turbid urine and apply heat to the upper part. If the turbidity is due to the presence of urates, the heated portion of the urine immediately becomes clear.



Fig. 42.—Urate of Soda and Crystals of Uric Acid (*h*), Oxalate of Lime (*o*), and Cystin (*c*). × 350.

2. To some urine in a test-tube add some liquor potassii, when the turbidity due to urates promptly disappears.

Microscopic Appearance of Urates.—The uratic deposit is composed of fine, somewhat regular granules, usually occurring in groups; sometimes the granules show spiny projections. (See Fig. 42.) Ammonium urate occurs only in alkaline urine, and is generally accompanied by a copious precipitation of triple phosphates. Ammonium urate appears as opaque brownish-red spherules with or without projecting spines.

Significance.—The excess of urates is of no special importance; they are increased in most conditions, accompanied by fever, and in many disturbances of metabolism.

PLATE VII



Uric Acid Crystals with Amorphous Urates. × 450.
(After Peyer.)

HIPPURIC ACID.

Hippuric acid, in combination with alkaline bases, is a normal constituent of the urine. The average quantity eliminated in twenty-four hours is one gram. This amount may be increased by exercise, by a vegetable diet, and by ingestion of benzoic acid. In cases where the total excretion of urine is greatly diminished, hippuric will be spontaneously thrown out of solution. This is, however, a rare occurrence, because hippuric acid is readily soluble in water.

Microscopic Appearance.—The crystals are characteristic rhombic prisms, resembling in a measure the coffin-lid crystals of triple phosphates. Hippuric acid crystals may, however, be distinguished by the fact that they are precipitated in acid urine only, and also because they do not dissolve on the addition of acetic acid. Phosphates, on the other hand, are precipitated only in neutral or alkaline urine, and are readily soluble in dilute acetic acid.

CREATININ.

This normal urinary constituent is present in the twenty-four hours' specimen to the amount of one gram. Creatinin is derived from the creatin of muscle. It is distinguished in the urine by its union with mercuric chlorid, with which salt it forms insoluble compounds. Similar characteristic compounds are formed with zinc chlorid and silver nitrate. The zinc chlorid combination has a characteristic appearance, by which this substance may be recognized. Under the microscope the zinc chlorid combination of creatin appears as minute needles arranged in balls and rosettes. Creatinin reduces alkaline copper solutions, and therefore affects, in a slight degree, the accuracy of the quantitative sugar estimations which depend upon the reducing power of sugar-containing urine.

Weyl's Test.—Add a few drops of a very dilute aqueous solution of freshly dissolved sodium nitroprussid and a few drops of dilute sodium hydrate solution to 8 to 10 cubic centimeters of urine in a test-tube. In the presence of creatinin a ruby-red color appears, which changes after a short time to an intense yellow. If this solution be heated with a little glacial acetic acid, the yellow will change to green and finally blue. Acetone

gives a similar reaction, but on the addition of acetic acid changes to a purplish red instead of green. If the urine be heated previous to the application of this test, the acetone will be driven off. This test is sensitive to about 1 part in 1700.

Jaffé's Test.—To the urine to be tested, add a few drops of a saturated watery solution of picric acid and a few drops of 10 per cent. sodium hydrate solution. If creatinin be present a red color appears immediately, which increases in intensity and remains permanent for a long time. If glacial acetic acid be added the color becomes yellow. Acetone gives a reddish-yellow color of less intensity than that produced by creatinin. Glucose, if present, may give a red color, especially if the mixture be warmed. This test is positive for 1 part of creatinin in 5000.

OXALIC ACID.

Oxalic acid is normally present in the urine only in very small amounts and it appears in combination with calcium as calcium oxalate. The normal amount of this substance in a urine of normal volume is held in solution by the acid sodium phosphate. Since oxalic acid requires 500,000 parts of water to effect solution of 1 part calcium oxalate, even the slightest variation in the normal relation results in its appearance as a deposit. Artificially the crystals may be thrown down by carefully neutralizing the specimen with dilute ammonia water, or by simply allowing the urine to stand for a time exposed to the air. The sediment of calcium oxalate is usually so scant that it is only recognized by the aid of the microscope. The more rapid the formation of these crystals in the urine after voiding, the smaller will the individual crystals appear; this will, in a way, indicate whether or not the presence of the crystals is due to a pathologic increase in the oxalic acid content, or merely to a change of reaction (loss of acidity) from standing.

Microscopic Appearance.—Oxalates are recognized in one of two forms: perfect octahedra, or in hour-glass and dumbbell forms. (See Plate IX, *a* and *b*, and Fig. 42.)

Significance.—These deposits sometimes follow the eating of stewed rhubarb or other acid fruits containing oxalates. Usually their existence in freshly voided urine indicates imperfect oxidation of retarded metabolism within the economy.

QUANTITATIVE DETERMINATION OF OXALIC ACID.

Baldwin's Method.—Five hundred cubic centimeters of the twenty-four-hour specimen are mixed with 1250 cubic centimeters of 95 per cent. alcohol in order to precipitate the calcium oxalate. This mixture is set aside for forty-eight hours, and then filtered, care being taken to remove the crystals from the walls of the beaker by a rod the tip of which is protected by a rubber tube. The sediment is washed thoroughly in a small beaker and treated with a few cubic centimeters of dilute hydrochloric acid. The filter is then washed with hot water until there is no further acid reaction, and the washings are collected and evaporated to 20 cubic centimeters. A little calcium chlorid solution is added to insure an excess of calcium. The hydrochloric acid is neutralized with ammonia, and then the solution is rendered slightly acid with acetic acid. Strong alcohol is added in an amount equal to one-half the volume of the fluid, and the whole is set aside for another forty-eight hours. The sediment of calcium oxalate is collected on an ash-free filter, washed with cold water and with a 1 per cent. acetic acid until free from chlorids; the filter is incinerated, cooled, and weighed. The ash is calcium oxid, each gram of which represents 1.6 grams of oxalic acid.

AMMONIA.

General Considerations.—This substance, although chemically belonging in the class of inorganic compounds, is so closely related to the nitrogenous metabolism that it is best discussed under this heading.

Ammonia is one of the most important products of protein metabolism. It is constantly present in small amounts in normal urine, averaging about 0.85 gram of NH_3 in twenty-four hours, representing from 4 to 5 per cent. of the total nitrogen. It is present in combination with various acids and may represent largely a portion of the nitrogen which has not been transformed into urea, but has been used to combine with acid substances formed in the protein metabolism of the body. Any increase in the production of acid in the system or any increased intake of non-carbonate-forming acids will lead to an increased

excretion of ammonium salts. This is an important factor in the metabolism of conditions associated with acidosis.

Significance.—The total output of ammonia will vary ordinarily with the diet; that is, proportionate to the intake of total nitrogen. While the increase of the total nitrogen of the urine on increased nitrogen intake is largely in the form of urea, yet a small increase in the absolute amount of ammonia must occur. Likewise we may observe a diminished intake of nitrogen, which, while reducing the absolute amount of ammonia, yet increasing it relatively. Thus, Folin finds that with a total excretion of 16 grams of nitrogen, an ammonia output of only 0.85 gram (4.3 per cent.), while on a nitrogen-free diet a total nitrogen output of 3.6 grams was observed, with an ammonia elimination of 0.51 gram (11.3 per cent.).[5]

Quantitative Method of Schlosing.—This method is most commonly used, and is here given, as it is fairly simple, though open to the objections that it is time consuming and does not yield absolutely accurate results.

Technic.—Twenty-five cubic centimeters of urine are placed in the vessel (preferably a Petri dish). Above this is placed a glass triangle upon which rests a dish containing 20 cubic centimeters of tenth-normal sulphuric acid. Twenty cubic centimeters of milk of lime are then poured into the dish containing the urine and the whole covered with a bell-jar, the borders of which have been well greased to make an air-tight union when the jar is placed upon the glass plate. This apparatus is then allowed to stand at room temperature from four to five days, during which time the ammonia, liberated by the action of the milk of lime upon the ammonia salts of the urine, will be taken up by the sulphuric acid in the vessel. At the end of this time the bell-jar is removed, the acid titrated with tenth-normal sodium hydrate, and the number of cubic centimeters of remaining acid determined. One cubic centimeter of tenth-normal sulphuric acid neutralized by the evolved ammonia represents 0.001704 gram of ammonia. This figure is multiplied by 4 to obtain the percentage ammonia value. If any moisture is present on the inside of the bell-jar it should be washed into the sulphuric acid before titration.[6]

[5] Webster's "Diagnosis."
[6] *Ibid.*

Formalin Method.—This method, originated by Ronchese and Malfatti, depends on the fact that a solution of an ammonium salt, treated with formaldehyd, decomposes with the formation of hexamethylenetetramin, the acid combined with the ammonia being liberated. This can then be determined by titration.

Dilute 10 cubic centimeters of urine with 50 cubic centimeters of water, add 2 or 3 drops of a 1 per cent. alcoholic solution of phenolphthalein, and neutralize with $\frac{n}{10}$ NaOH. Five

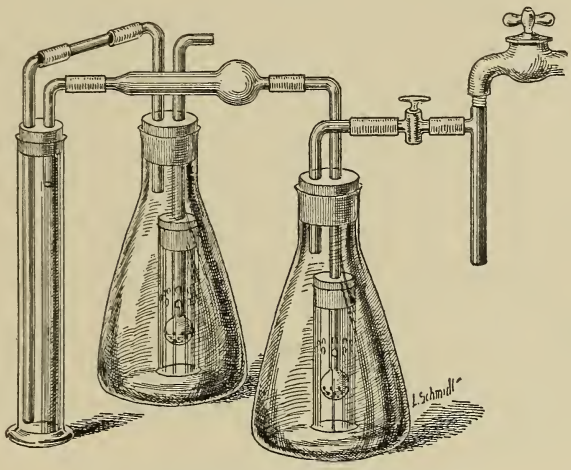


Fig. 43.—Folin's Ammonia Apparatus.

cubic centimeters of formalin, previously neutralized with $\frac{n}{10}$ NaOH, are added to the neutralized urine and the mixture is again titrated with $\frac{n}{10}$ NaOH to the appearance of a faint permanent pink color, the amount of alkali used being noted. It is evident that 1 cubic centimeter of this $\frac{n}{10}$ NaOH is equivalent to 1 cubic centimeter of $\frac{n}{10}$ NH_3, or, in other words, represents 0.001704 gram of NH_3. Multiply the number of cubic centimeters of $\frac{n}{10}$ NaOH used by this factor to obtain the NH_3 in 10 cubic centimeters of urine.

Caution.—It has been found that formalin combines, also, with the NH_2 group of the amino-acids, thus leading to results higher than those of the Folin method. If the figure obtained

by the Folin method be subtracted from that of the formalin method, the result is the NH_2 referable to amino-acids.

Folin's Method.—This method, while giving most accurate and satisfactory results, is considered by the writer as too difficult and cumbersome for routine use by the average practitioner. Full explanation of the technic will be found in the larger works on chemical diagnosis.

CYSTIN.

This substance, in exceedingly small amounts, is a normal constituent of urine. It is almost insoluble in water, and in the very rare cases where it is present in increased amount it is deposited. To the naked eye the deposit is abundant and somewhat resembles that of urates. It is, however, not dissolved by heat or by the vegetable acids, but is readily dissolved in ammonia water. The ammoniacal solution exposed on a slide and examined under the microscope slowly develops crystals in the form of hexagonal plates. (Plate X, *d*.) Iodoform, which has found its way into the urine from surgical dressings, may be mistaken for cystin.

Tests.—When urine containing cystin undergoes decomposition, it develops the odor of hydrogen sulphide. When boiled with a solution of lead oxid in sodium hydrate, black-lead sulphid is formed.

LEUCIN AND TYROSIN.

These are present in the urine in cases of acute yellow atrophy of the liver, typhoid fever, and phosphorus poisoning. (Plate X, *e* and *f*.)

To examine for these substances, the urine must be concentrated on a water-bath, and the cooled liquid examined under the microscope.

Microscopic Appearance.—Leucin appears as greenish-yellow spheres with concentric markings or radiating spines. Tyrosin as feathery sheaves, which resemble the frayed end of a rope.

PART II.

ABNORMAL CONSTITUENTS OF THE URINE.

ALBUMIN.

General Considerations.—Albumin is probably present in minute quantities in all urine, since urine always contains a variable number of cellular elements derived from the urinary tract. Thus, even under normal conditions, by means of sufficiently delicate tests, we can always demonstrate the presence of albumin. This amount is, however, so small that it returns a negative result with the tests commonly employed in the clinical laboratory for the detection of albumin in urine. Therefore the presence of demonstrable amounts of albumin, by the tests now to be described, must be considered as variations from the normal. The "normal" albumin is *nucleo-albumin,* and its clinical significance is slight, even when present in an appreciable quantity.

Several other albumins are found in the urine, of which *serum-albumin* and *serum-globulin* are of the greatest clinical importance, and the term albuminuria is understood to indicate the presence of one or the other, more often of both, in the urine. Other albumins which may be found in the urine, besides those referred to above, are albumose and fibrin.

The diagnostic and clinical importance of *albumosuria* have not yet been determined. Traces have been found in many of the infectious fevers. A large albumosuria may be significant of multiple myelomata, since in this affection large amounts are frequently observed. It has also been found in osteomalacia and in nephritis.

Fibrin, when present, indicates the direct entrance of blood-plasma into the urinary tract. It must be distinguished from blood-clots, which come under the head of hematuria.

Causes of Transient Albuminurias.—Physiologic or Functional Albuminuria: The presence of easily demonstrable amounts of albumin can hardly be considered normal under any circumstances. However, the presence of a functional albu-

minuria is recognized by some authorities. An albuminuria does, however, occur, in which the organic change in the kidney, if there be any, is so slight and evanescent as to be unimportant. This form of albuminuria, compared with that caused by severe and permanent kidney change, may, with propriety, be termed functional, but never, physiologic.

Functional.—Albuminuria may occur after violent exercise, after a cold bath, after severe mental strain, and after overeating of proteid food-stuffs, particularly eggs.

Whether or not the disturbance giving rise to the albuminuria is insignificant and transient (renal hyperemia or anemia), or partakes of the gradual and progressive variety due to permanent alteration in kidney structure, to which the term chronic nephritis is applied, must be determined by the history, symptoms, and progress of the case.

Fevers.—Here the occurrence of albumin in the urine is, in all probability, dependent largely upon the intensity of the infective process, causing irritation of the kidney epithelium, incident to the passage of toxic substances circulating in the blood, through the glomeruli and tubules of the kidneys; the inflammatory process so induced being indicated by the amount and duration of the albuminuria.

Specific Blood-Changes.—The nature of the changes in the blood occurring in scurvy, peliosis, purpura, hemophilia, and pernicious anemia, is obscure in relation to the production of albuminuria.

Foreign Substances in the Blood.—This form of albuminuria is seen after the excessive ingestion of lead, mercury, ether, chloroform, etc. These probably produce the albuminuria by irritation of the kidney through efforts of that organ at elimination of the substances themselves, or their oxidation products.

Circulatory Disturbances.—Here the passive congestion occurring in cardio-vascular and pulmonary diseases directly affects the permeability of the renal epithelium.

Neurotic.—This term applies to albuminuria occurring during or immediately succeeding attacks of apoplexy, migraine, tetanus, delirium tremens, and certain head injuries. It is probably toxic in origin.

Extra-Renal.—The presence of blood, pus, chyle or lymph, which has entered the urinary tract in appreciable quantities, will cause albumin to appear in the urine. Recently attention has been called to a form of albuminuria resulting from abnormalities in the function of the genital tract. This form comes and goes irregularly, and does not show the presence of spermatozoa.[1]

In the majority of the above conditions the quantity of albumin is small and varies from day to day. Under these circumstances the relative clinical importance of the finding will be determined by microscopic examination of the urine, aided by the history and course of the disease in which it occurs.

These small and transient albuminurias are of considerable importance from the standpoint of life insurance, since too much importance is frequently placed on the finding of a trace of albumin, particularly in a single specimen, which is usually voided succeeding a full meal.

Qualitative Tests for Albumin.—In detailing the following methods for determining the presence of albumin in the urine, no attempt will be made to offer a great variety of tests. The principle involved in the majority is the same, and depends on the coagulation of albumin in the specimen by heat or by chemical reagents.

Before proceeding to make the tests, the urine must be filtered or centrifugated to remove the turbidity. If this is due to large numbers of bacteria it cannot be entirely removed by this process. The persistence of a bacterial turbidity will interfere with the delicacy of the tests.

1. Boiling and Acetic Acid.—This is the best and simplest test for routine laboratory work. If albumin is found it is well to corroborate the finding by another method. The urine should not be alkaline in reaction.

The Test.—Fill a narrow test-tube almost to the top with clear urine, boil the upper third, being careful to avoid too much agitation. In the presence of albumin, or the neutral earthy phosphates, or of both, a white cloud will appear in the heated area. Now, upon the addition from a pipette of a few drops of

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[1] William Glenn Young: New York Med. Jour., Jan. 5, 1907.

10 per cent. acetic acid, the cloud, if due to phosphates, will vanish, while if caused by albumin it will remain and will perhaps be increased in density. The chief advantages of this method are its simplicity and delicacy, and the fact that it allows a comparison between the treated and untreated urine in the same tube. The test is most satisfactory when performed in strong daylight with the tube held close to a dull-black background.

Caution.—At times the addition of more acid to a urine already acid will convert the albumin into an acid albuminate, which is not coagulable by heat. To overcome this the acetic acid should always be dilute (10 per cent.), and should never be added to acid urine before boiling, and then only in sufficient quantity to produce the desired reaction.

2. Heller's Test.—This test is best performed with the Meeker albumin tube.

The Test (with the Meeker tube).—Allow about one-half inch of warm, clear urine to enter the tube through the small curved top. Place the forefinger over the upper end of the tube to retain the urine, and dry with care the outside of the tube; plunge it immediately into nitric acid of sufficient depth to allow the acid to flow in and elevate the urine above it. If this is done with care the line of demarkation between the urine and the acid will be very sharp, and the presence of albumin will be shown by a white line at the plane of contact. Five minutes should be allowed for the appearance of the reaction, the delicacy of which is increased if both the urine and the acid are previously warmed to about 60° C. (a temperature too high to be endured by the hand for more than a few seconds).

A roughly quantitative test by Heller's method is suggested by D. W. Prentiss.[2]

Technic.—1. Make the underlying nitric test for albumin, in a test-tube.

2. Allow the tube to stand two minutes.

3. Hold the tube between a black or dark object and the eyes, on the level with the eyes.

4. Note the exact thickness of the ring of albumin at the line of contact.

[2] Med. Council, Philadelphia, August, 1912.

5. On the chart draw parallel vertical lines like this: | | which represent the test-tube. Connect these lines by a cross line the exact breadth of the thickness of the coagulated albumin.

Example: |—| line of coagulated albumin exact thickness.

Example of diminution in albumin in 5 examinations in a case of toxemia of pregnancy (see Fig. 44) :—

Sources of Error.—If a cloud appears at the line of contact in a urine known to contain an excess of urates, this source of error can be eliminated by diluting the urine with one or two volumes of water, and repeating the test.

Nitric acid precipitates certain resins which appear in the urine after it has been administered for medicinal purposes. If these cannot be excluded another test should be employed.

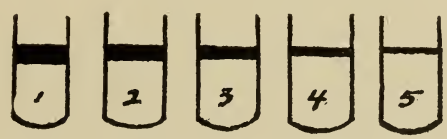


Fig. 44.—Method of Roughly Recording Albumin in Urine.

If an excess of phosphates exist they may be held in solution by the addition of a few drops of a 10 per cent. solution of acetic acid.

Nucleo-albumin may be held in solution by the addition of one-sixth volume of saturated solution of NaCl, and then proceeding as before.

Purdy's Test.—Mix a half-inch of Purdy's reagent (see Appendix) with four inches of urine in a six-inch test-tube; if a whitish cloud appears either at once or after standing, the urine contains albumin.

Tanret's Test.—Acidify a quantity of urine with dilute acetic acid; if mucin produces a cloud, filter and to the filtrate add about 5 cubic centimeters of alcohol and heat slightly. Now substitute this prepared urine for the urine used in the description of the nitric acid contact-test, using Tanret's reagent in place of the nitric acid (see Appendix) ; the presence of a white line at the level of contact denotes albumin.

Ulrich's Test.—The specimen should always be filtered. The reagent used is a saturated solution of common salt, acidified

with 1 per cent. acetic acid. A few cubic centimeters are poured into a test-tube and boiled. The urine is then allowed to flow over the hot acid brine from a pipette, forming a sharp line of contrast. The hot brine will coagulate the albumin, while the acid will prevent the precipitation of the phosphates. The presence of salts prevents the precipitation of "nucleoalbumin."

Caution.—The phosphates are sometimes precipitated by this method, and it is best to add to the urine as a routine measure about one-sixth volume of the acid salt solution before applying the test.

Quantitative Estimation of Albumin.—Method of Esbach: For clinical purposes as a ready means of comparing the albumin content in a given case or a number of cases, this method will be found applicable and convenient.

The amount of gravitated precipitate should never be confounded with the actual percentage of albumin as determined by accurate weighing methods, which rarely amounts to more than 4 or 5 per cent., while by the method of Esbach the moist precipitate may exceed half of the bulk of the fluid in the tube.

The Test.—Fill the Esbach albuminometer tube (see Fig. 45) with urine to the line marked "U," and fill with Esbach reagent (for preparation see Appendix) to the line marked "R." The tube is then closed with a rubber stopper, and the liquid thoroughly mixed by repeated inversion without shaking, and is then set aside in a vertical position for twenty-four hours, when the layer of precipitated proteid will have settled to the bottom. The granulations indicate the grams of proteids in the liter. If the amount of proteid is large, it is advisable to dilute the urine with two or more parts of distilled water before beginning the test, and then to correct the final reading by the dilution. To insure accuracy the tube should stand in a temperature of about 15° C. (60° F.). Amounts of albumin less than 0.05 per cent. will not sediment, so cannot be estimated by this method.

Modification of the Esbach Test.—The great drawback has always been the necessity of waiting twenty-four hours for complete precipitation before reading the result. This method differs from Esbach's simply in the addition of 10 drops of a 10 per cent. ferric chlorid solution to the measured amount of urine after it is put in the tube and before the Esbach solution

is added, and after gentle mixing place the tube in a water bath at a temperature of 72° C. The precipitation begins almost immediately and is complete in a few minutes, when the results are read in the usual manner. This method is but slightly more complicated than the usual Esbach estimation, and has been employed by the author in a series of specimens, using the old method for control. No differences were noted. It seems to be an accurate modification of much value in saving time.

Tsuchiya's Method.—The technic of this test is similar to that of Esbach, except that a solution of phosphotungstic acid in acidulated alcohol is substituted for the Esbach reagent.

Normal urine gives only a faint unreadable precipitate, while small amounts of albumin as well as large ones are completely precipitated by it. This albuminous precipitate settles regularly and quickly, and includes the other urinary albuminous substances (see below), foaming or floating of the precipitate being rarely seen. Careful observers have shown that this method yields fairly accurate results for comparative purposes, and that the figures agree closely with those of the gravimetric methods.[3]



FIG. 45.—IMPROVED ESBACH ALBUMINOMETER.

Albumosuria.—Albumose appears in the urine as a part product of proteid metabolism. It is a pre-peptone which appears in the blood and can be produced by artificial digestion. Pathologically, its continued and marked presence in the urine usually denotes multiple myelomata. It has also been noted in syphilis, pneumonia, peritonitis, and cholera.

THE TEST.—Add concentrated nitric acid to the urine, agitate, and allow to stand in a cool place. A heavy precipitate will occur, which upon heating disappears, only to reappear on cooling. At the same time the mixture gradually assumes an intense yellow color.

Albumin of Bence-Jones.—This substance has been repeatedly found in cases of multiple myelitis. Its exact nature is

[3] It is advisable to note that at this point, in speaking of per cent. of albumin, one should, to be correct, refer only to the number of grams of albumin by weight in 100 cubic centimeters of urine. It is not infrequent to hear of case histories in which 50 to 75 per cent. of albumin is reported. This means only per cent. by volume and should be so stated, as it has no clinical significance. Actually the urine very rarely contains more than 5 per cent. of albumin.

not definitely known, but it is generally regarded as an albumose. Its reaction to heat and nitric acid is similar to that of albumose. The test for its positive identification is complicated and difficult, and will hardly be required of the clinical laboratory worker.

Nucleo-Albumin.—Nucleo-albumin, beyond a faint trace, will give the same reactions as true blood-albumins. Its presence may be inferred when there is an albumin reaction in a specimen which shows an absence of renal elements with an excess of epithelia of bladder and urethral type.

TEST.—To dilute acid urine add an excess of acetic acid, stand aside, and gradually a faint cloud will appear.

DIFFERENTIAL TEST.—If the preceding test gives a positive reaction, take a fresh portion of the same specimen, and first add one-sixth volume of a saturated sodium chlorid solution, and then some dilute acetic acid. The presence of sodium chlorid will prevent the precipitation of nucleo-albumin in the last test.

Fibrin, when present in the urine, usually occurs in sufficient amounts to form clots, which are easily distinguishable. When the amount is small it may become necessary to determine the presence of this substance by chemical means.

TEST FOR SERUM-GLOBULIN.—Add to some urine contained in a small beaker sufficient ammonia water to produce a slightly alkaline reaction; a cloud of phosphates will appear and will settle to the bottom. As soon as a layer of clear urine appears, decant this into a test-tube. Mix this clear urine with an equal quantity of a clear saturated solution of ammonium sulphate. If a flocculent precipitate appears in a few minutes the albumin present is serum-globulin.

TEST FOR SERUM-ALBUMIN.—Take the final solution obtained in the foregoing test and filter it free from serum-globulin. Heat the filtrate to boiling, and add one-tenth volume of strong nitric acid. If a precipitate now appears the urine contains serum-albumin.

Proteose.—Mix about 50 cubic centimeters of urine contained in a beaker with 5 cubic centimeters of strong nitric acid. Heat the solution rapidly to about 80° C. If a precipitate appears, prepare a hot filter-paper by pouring boiling water through it in a funnel. Now filter the hot mixture of

urine and nitric acid. Add to the clear, hot filtrate its own volume of a clear, saturated solution of sodium chlorid. Allow the mixture to cool. If any precipitate appears in this cooled mixture then proteose was present in the specimen of urine under examination.

GLUCOSE.

General Considerations.—A trace of sugar can at times be detected in apparently normal urine from healthy subjects by special tests of great delicacy. The ordinary tests employed in clinical medicine, however, do not react to these minute amounts of sugar, hence the findings of glucose by the tests about to be outlined must, in every case, be considered pathologic.

The reducing power of normal urine is, in part, due to the activity of uric acid and creatinin, and is equal to about a 0.1-per-cent. solution of glucose.

Under pathologic conditions the reducing power of urine may be enormously increased, due to the abnormal presence of glucose. This may be present up to as much as fifty grains to the ounce (10 per cent.).

The detection of sugar in the urine is based upon a knowledge of the following properties of glucose:

1. In hot alkaline solutions, reduces the oxides of copper and bismuth.

2. With brewer's yeast, ferments, forming alcohol and carbon dioxid.

3. Enters into chemical combination with phenylhydrazin to form characteristic insoluble crystals of glucosazone.

4. Deflects polarized light to the right.

In the application of all tests but the fermentation-test, it is necessary to use urine free from albumin. If this be present it must first be removed by the addition of dilute acetic acid and boiling to cause precipitation. This is then filtered out, and the filtrate used in the tests for sugar.

Reduction Tests.—Method of Fehling[4] (for preparation of reagent see Appendix): The sample of urine to be tested is first examined carefully for the presence of albumin. If this be

[4] The suggestions for the performance of this test are taken from a communication by C. W. Louis Hacker, M.D., appearing in the Jour. Amer. Med. Assoc., page 252, Jan. 25, 1908.

found it must be removed by heat and acetic acid, and the filtrate used for the test. About 4 cubic centimeters of freshly prepared Fehling's solution are diluted with three parts of water, and brought just to the boiling point in a clean test-tube. Immediately two drops of proteid free urine are added, and the tube shaken vigorously. If the urine contains more than 2 per cent. of sugar, a yellowish-red precipitate immediately appears, changing rapidly to a brownish-red or bright red, which settles out slowly, leaving a greenish-blue supernatant fluid. If the reaction be positive, it is better to roughly dilute the urine with two or four parts of water, and to perform the test again.

With the strengths of dextrose from ½ to 2 per cent., two drops of urine, as stated above, will cause a characteristic precipitation of red suboxid of copper, about which there can be no doubt. With smaller percentages the change may come slowly, but even then heat need not be applied after the addition of the urine. Here little change may be seen in the Fehling's solution by transmitted light, but by reflected light a brownish-red tint is evident, which finally develops into a decided light-red precipitate, gradually settling out and leaving a clear, blue, supernatant fluid. The pentoses, lactose, and even maltose, by this method, do not give the characteristic reaction. Urines showing this reaction always give a reaction with Nylander's solution (see next page).

If no change occurs in the Fehling's solution after the addition of the two drops as above, the solution may then be warmed just to the boiling point, and from two to four drops of urine added and the mixture observed. This should be repeated until in all twenty drops of urine have been added. If no change occurs at this point, the urine does not contain dextrose to the extent of 0.5 per cent.

Cautions.—The disadvantages of using large amounts of urine (an equal or even one-half volume of the Fehling's solution) are two-fold. In the first place, the strongly alkaline copper solution with urine of high specific gravity throws down a more or less voluminous precipitate of cupric phosphate, which takes out the blue color of the solution, and changes it to a dirty, dark green. In the second place, the alkaline solution, acting on the ammonium salts in the urine, liberates sufficient ammonia to

hold in solution small amounts of the red suboxide of copper, obviously interfering with the detection of traces of dextrose. Very frequently under these conditions, and especially if the solution has been boiled, even for a few seconds after the addition of the urine, the greenish solution slowly takes on a dirty, yellow-brown color, then become turbid, and, eventually, on standing, there separates out a finely divided opalescent, greenish-yellow precipitate, which only settles out completely after the tube has been allowed to stand overnight. In certain cases this reaction does not begin until a few seconds have elapsed after the addition of the urine. Such a reaction may be termed a pseudo-reaction.

Fallacies.—Neglect of the above precautions will result in the occurrence of the pseudo-reaction in about 5 per cent. of normal urines examined.

The following substances, if present in the urine in more than normal amounts, will result in partial reduction of Fehling's solution. Uric acid, creatinin, hippuric acid, mucin, hypoxanthin, and alkapton. Also the presence of the oxidation products of the following drugs: The alkaloids, arsenic, carbolic acid, and hexamethylenamin (urotropin). The latter substance does not ordinarily reduce bismuth.

De Jager's Test.[5]—De Jager describes the following as an improvement in his older test for sugar in the urine: Milk of lime, made by adding 30 grams of calcium hydroxide to water, up to 100 cubic centimeters, is allowed to stand at least twenty-four hours before it is employed in the test. To 5 cubic centimeters of the urine add 10 drops of milk of lime and 5 drops of a 10 per cent. solution of copper sulphate. The mixture is then heated to boiling. Where sugar is present, a red or violet precipitate appears, unless the amount of sugar is very large, when a yellow color is seen. The limit of sensitiveness of the test is 1 part of sugar in 10,000. The lime suspension should always be well shaken before it is used.

Benedict's Test.—This is a modification of Trommer's test.[6] The reducing action of glucose in alkaline solution is diminished by strong alkalies, such as the hydrates. This prop-

5 Zentralblatt für innere Medizin, June 22, 1912.
6 Münch. med. Wochen., No. 27, 1912.

erty may prevent such well-known solution as Fehling's and Haines's (discussed below) from demonstrating small quantities of sugar.

This solution of Benedict's (see Appendix) is more sensitive (about 10 times) to urinary sugar than are the other copper solutions. Moreover, it is not appreciably reduced by creatinin and uric acid, nor by such preservatives as chloroform, chloral, or formaldehyd, which may become a source of error in the other tests. It is, however, promptly reduced by the alkapton acids and glycuronic acids.

The Technic.—Five cubic centimeters of the reagent are placed in a test-tube to which not more than 10 drops of urine are added. The mixture is then heated to boiling for from one to two minutes and allowed to cool spontaneously. In the presence of glucose the entire body of the solution will be filled with a precipitate, which may be red, yellow, or greenish in tinge. If the quality of glucose be low (under 0.3 per cent.) the precipitate forms only on cooling. If no sugar is present, the solution either remains perfectly clear or shows a faint turbidity, which is blue in color and consists of precipitate urates. This test may be especially recommended for all qualitative work, as it is very delicate and has few fallacies.

Haines's Test.—Haines has introduced a modification of Trommer's test by adding glycerin instead of Rochelle salt, to increase the amount of copper in solution. This test is much more convenient than Fehling's, the solution having the advantage of keeping almost indefinitely. It is, however, far less delicate than is Benedict's and is reduced by preservatives as well as by excess of many normal urinary constituents, especially by uric acid and creatinin.

To test, one or two cubic centimeters of this solution (see Appendix) are placed in a test-tube and gently boiled. Six drops of the suspected urine are added and the upper portion of the mixture brought to a boil and immediately removed from the flame. If sugar be present, an abundant yellow or yellowish-red precipitate is thrown down; if no such precipitate occurs, sugar is absent.

Caution.—The precautions to be observed in using this test are: never to add a total of more than 10 drops of urine and not

to boil the mixture for more than one or two seconds after the addition of the urine.

Trommer's Test.—To half-inch of urine in a test-tube, add one inch of liquor potassii and a few drops of a 10 per cent. solution of copper sulphate sufficient to cause blue discoloration with slight cloudiness. Heat this mixture to the first signs of boiling. If sugar be present the copper will be reduced to yellow or red oxid. Slight precipitations, which may occur after prolonged boiling, are no proof of sugar.

Delicacy.—This test, if carefully performed, will demonstrate the presence of sugar in 0.025 per cent. solution.

Bottger's Bismuth Test.—Urine containing coagulable proteids must first have them removed by heat and acetic acid. Mix in a clean test-tube equal volumes of urine and liquor potassii, and add a few grains of bismuth subnitrate and boil. In the presence of glucose the white powder in the bottom of the tube will gradually become gray, brown, or black.

Nylander's Bismuth Test.—Nylander modifies the preceding test by substituting for the subnitrate a special reagent containing bismuth (for reagent see Appendix). Albumin, if present in the urine, must be removed in the usual way. To 10 cubic centimeters of Nylander's reagent, add 1 cubic centimeter of urine and bring to the boiling point. A brown or black discoloration of the liquid denotes sugar.

Fallacies of bismuth tests. A slight reduction of bismuth may be caused by turpentine, eucalyptus, or the ingestion of large amounts of quinine. Albumin and sulphur compounds in urine produce a black precipitate.

Delicacy.—These tests show the presence of glucose in 0.025 per cent. solution.

Phenylhydrazin Test.—This test depends upon the production of characteristic crystals of phenylglucosazone by the combination of glucose and phenylhydrazin in hot solution, and their recognition with the aid of a microscope.

The Test.—Fill a beaker half full of water and warm upon the wire-gauze over a low Bunsen flame. Place a test-tube, containing the urine, which has been acidulated with a few drops of dilute acetic acid, in the beaker. While waiting for this to warm prepare the following solution (this must be made fresh,

as it does not keep well): Weigh out 1 gram of phenylhydrazin hydrochlorid and 2 grams of sodium acetate. Mix the salts and dissolve in 10 cubic centimeters of distilled water acidulated with 2 drops of dilute acetic acid. Filter to clarify. After the water in the beaker has boiled for five minutes, observe the urine in the tube; if it has become turbid, filter. To 10 cubic centimeters of hot urine in a clean test-tube, add 5 cubic centimeters of the filtered reagent, replace in a beaker of boiling water, and continue boiling for one hour. Then cool quickly by immersing the tube in cold water. If sugar was present in the urine a crystalline precipitate will appear. This should be taken up in a pipette and transferred to a microscope slide for examination. The characteristic crystals of phenylglucosazone are fine, faintly yellow needles arranged in the forms of fans, rosettes, and sheaves.

Fallacy.—Levulose, if present in the urine, will produce similar crystals; these can be differentiated by a comparison of the fusing points of the two compounds.

Phenylglucosazone fuses at 204° C.

Phenyllevulosazone fuses at 150° C.

Delicacy.—Glucose forms characteristic crystals when present in 0.025 per cent. solution.

QUANTITATIVE OR VOLUMETRIC DETERMINATIONS OF GLUCOSE IN THE URINE.

Method of Fehling.—Measure into a beaker or porcelain dish 10 cubic centimeters of accurately prepared Fehling's solution and 40 cubic centimeters of distilled water. Heat the mixture over wire-gauze to boiling. Prepare a dilute solution of suspected urine by adding 9 parts of distilled water to 1 part of urine. This mixture is placed in a burette. Now to the boiling solution add the dilute urine, a few drops at a time, from the burette. Continue adding, boiling, and stirring until the blue color of the Fehling solution completely disappears when viewed by transmitted light. Note accurately the number of cubic centimeters of urine used.

Fehling's solution is a standard solution of copper sulphate, 10 cubic centimeters of which is exactly decolorized by 0.05 gram of glucose.

Example.—Suppose 8 cubic centimeters of diluted urine were used in decomposing 10 cubic centimeters of Fehling's solution. Then, of undiluted urine, 0.88 cubic centimeter was required. This amount then contained 0.05 gram (10 x 0.005) glucose. To calculate the percentage of sugar in the sample of urine: 0.8 : 0.05 : : 100: X, which, in this case, "X" equals 6.25 per cent. To calculate the grams of glucose voided in twenty-four hours: If 1500 cubic centimeters of urine were voided in twenty-four hours, then 0.8 : 0.05 :: 1500 : X, which equals 93.75 grams glucose.

Caution.—The dilute urine should be added very slowly with frequent boilings and examinations by transmitted light to avoid passing the end reaction.

Fallacies.—The sources of error in this test are the same as those given under Fehling's qualitative test on page 269.

Bang's Method.—The principle of this method is as follows: The urine is treated with an excess of standard alkaline copper solution (see Appendix, page 403) and boiled to exhaust the glucose present by reducing part of the copper content. The amount of copper remaining in excess is then determined by titration with a solution of hydroxylamin sulphate, and from this the amount of sugar is calculated. (For reagent, see Appendix.)

Technic.—Ten cubic centimeters of urine (5 or 2 cubic centimeters diluted with water to 10 cubic centimeters if more than 0.6 per cent. of sugar is present) are measured into a 200 cubic centimeter Jena-Erlenmeyer flask and treated with 50 cubic centimeters of the copper solution. Heat on a wire gauze to boiling for exactly three minutes. Cool quickly to room temperature by immersion of the flask in cool water. Now titrate with the hydroxylamin solution until a colorless mixture results. From the number of cubic centimeters of hydroxylamin sulphate solution used, calculate the sugar in milligrams by means of the table. A simple calculation yields the percentage and total amounts. (See Appendix for table, page 439.)

PURDY'S QUANTITATIVE METHOD.

Into a beaker or boiling flask of 250 cubic centimeters' capacity put 35 cubic centimeters of Purdy's reagent (see Appendix) and 70 cubic centimeters of distilled water. Boil

steadily over a wire gauze, and add urine slowly from a burette until the blue color begins to fade; now proceed cautiously, and after the addition of each drop wait for a few seconds to see if the end-reaction is complete. Continue adding until the solution of the reagent is colorless and transparent. To obtain this result the total amount of urine employed must have contained 0.02 gram of glucose.

Example.—Suppose the amount of urine employed to completely decolorize the reagent was 4.5 cubic centimeters, then 4.5 cubic centimeters of urine contained 0.02 gram of glucose; or 1 cubic centimeter of urine contains 0.0044 gram of glucose. This multiplied by 100 will give the percentage of glucose which is equal to 0.44. If the color of the reagent is changed by less than 4 cubic centimeters of urine, it is best to dilute the urine with one or two parts of water, and then multiply by this factor to obtain the final result.

This method is rapid, the technic is simple, and the end-reaction definite and sharp. The reagent is stable.

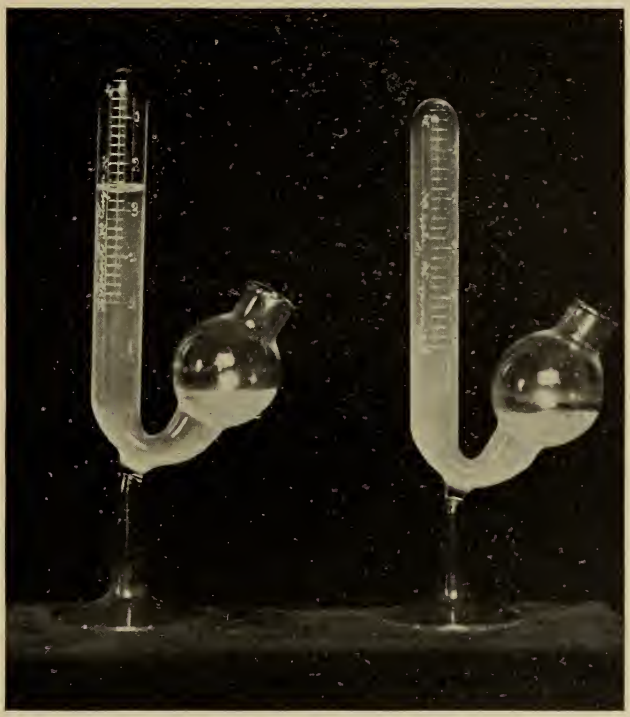
FERMENTATION SACCHARIMETER.

None of the substances found in the urine, which give the reduction reactions resembling glucose, are susceptible of alcoholic fermentation. This fact establishes the accuracy of the test. The test itself depends upon the fact that when sugar-containing urine is mixed with a quantity of brewers' yeast and kept in a warm place for twenty-four hours, it will ferment, giving off bubbles of CO_2, and at the same time forming alcohol.

By measuring the amount of carbon dioxid gas formed during this fermentation process, we are enabled to estimate the percentage and amount of sugar contained in the specimen under examination. A specially shaped and graduated tube (see Fig. 46) is usually employed in this test. It is known as the Einhorn saccharimeter. The upright tube is graduated from its closed upper end downward from 0.1 to 1 per cent. It will not estimate sugar in amounts less than 0.1 per cent., and owing to the varying solubility of the various gases of the urine, depending on differing reactions and the density, it cannot be regarded as absolutely accurate.

The Test.—Urines containing less than 5 per cent. of glucose must be diluted five times with water. Urines containing more than 5 per cent. must be diluted ten times before proceeding with the test.

Mix in a large test-tube about 20 cubic centimeters of



A B

Fig. 46.—Einhorn Saccharimeter in Use.

A shows Gas formed after Twenty-four Hours from a Diabetic Urine (Dilution 1 to 10) containing 2.7 Per Cent. of Sugar; B, Control with Normal Urine.

properly diluted urine with one-twelfth of a cake of fresh compressed yeast. Completely fill the upright tube of the saccharimeter with this mixture, so as to exclude all air from the graduated tube. Fill a second saccharimeter with yeast dissolved in distilled water or with urine known to be free from sugar. These tubes are to be kept in a warm place and allowed to remain undisturbed for from eighteen to twenty-

four hours. By this time the sugar-containing urine will be found to have been displaced by gas in the vertical tube. The percentage corresponding to the level of the fluid will represent the percentage of sugar in the diluted urine. This figure, multiplied by the dilution, will represent the percentage of sugar in the specimen under examination.

Cautions.—The urine must be faintly acid, and must be so diluted that the specific gravity will be less than 1008, and when diluted must contain less than 1 per cent. of glucose. If any gas is liberated by the yeast or fluid in the control-test, this amount must be subtracted from that indicated in the test-mixture before computing the percentage.

The internal administration of mercuric chlorid, iodoform, salicylic acid, hexamethylamin, quinine, and other antiseptic drugs will inhibit fermentation, and therefore must be excluded before testing.

Robert's Differential Density Method.—This method depends for its result upon the alteration in density occurring from the fermentation of saccharine urine.

The Test.—Mix thoroughly about 120 cubic centimeters of urine with half a cake of compressed yeast. Take and record accurately the specific gravity of this mixture. Set aside in a warm place (thermostat preferred) for twenty-four hours, and at the expiration of this time again take the specific gravity and subtract the second reading from the first. Each degree of the remainder (showing density lost) represents approximately one grain of sugar to the ounce. To obtain the percentage of sugar in the specimen, multiply the degrees of density lost through fermentation by the factor 0.219.

Example.—Specific gravity before fermentation 1041
Specific gravity after fermentation 1023

Degrees of density lost 18

This is approximately the grains of glucose in each ounce of urine; 18 x 0.219 equals 3.94% glucose.

This test is easily performed, and is conclusive evidence of the presence of glucose. It is, however, not strictly accurate, and is not to be relied upon when the amount of glucose present is less than 0.5 per cent.

POLARIMETRIC METHOD.

Because of its ability to turn the ray of polarized light to the right, glucose is called dextrose. The accurate determination of glucose in urine may be made by those possessing a polariscope. The degree of dextro-rotation can be read on a graduated scale and calculated as percentage or grams of glucose.

Since albumin in solution deflects the polarized ray to the right, this must first be removed by acidulating, boiling, and filtering. To make an accurate estimation the urine must be

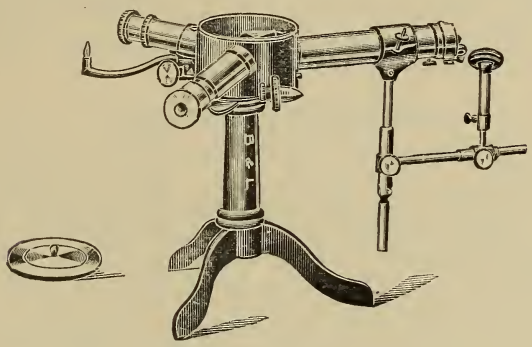


FIG. 47.—POLARIMETER. (A. H. T. Co.)

first decolorized by shaking with a piece of lead acetate and filtering.

DELICACY.—Instruments vary in delicacy. Usually distinct dextro-rotation may be detected when glucose is present in 0.025 per cent.

FALLACY.—Maltose, which rarely occurs in the urine, if present, will deflect the ray even more than will glucose.

(For description of technic see larger works on clinical chemistry or polarimetry.)

CLINICAL SIGNIFICANCE OF GLYCOSURIA.

The presence of glucose in the urine is pathologic. If the amount be abundant and persists, associated with copious water-drinking and eating, while the patient is at the same time emaciating, it is indicative of diabetes mellitus. The urine in this condition is usually pale with a fruity odor, and while over-

abundant, has a high specific gravity. As much as 10,000 cubic centimeters of urine may be excreted in twenty-four hours; at the same time the specific gravity may be maintained at 1050.

Temporary Glycosuria.—The appearance of small amounts of sugar may occur transiently in the urine after excessive ingestion of sugar; and reducing substances, such as glycuronic acid, may give a spurious positive reaction. Copper reduction by such substances is not corroborated by either fermentation or the phenylhydrazin tests.

Glycosuria also may accompany certain diseases of the brain, spinal cord, and pancreas, or be a transitory accompaniment of phthisis, pneumonia, cirrhosis, pulmonary edema, or cholera.

LEVULOSE (D-FRUCTOSE).

Levulose is found very widely distributed throughout the vegetable kingdom, especially in fruits. Honey is almost a pure levulose. It may be found in the urine, transudates, or exudates after a large intake of levulose-containing food or may occur spontaneously, even when the subject has taken little such food.

Levulose forms exactly the same phenylosazon as does glucose, so that it is a matter of great difficulty to differentiate those bodies by the ordinary tests (see page 272).

Seliwanoff's Test.—This test has been advanced as one characteristic for the ketones in distinction from the aldehydes. Ten cubic centimeters of urine are treated with a few crystals of resorcin and 5 cubic centimeters of concentrated HCl. If the mixture be warmed, a brilliant red color appears in the presence of a ketone (levulose), while no coloration is observed with an aldehyd (glucose). If the mixture be heated too strongly or too long, mannose and maltose may also give a positive test.

If the red solution formed in this reaction be neutralized with sodium carbonate and extracted with amyl alcohol or, preferably, with acetic ether (Borchardt), the extract will have a yellow color with a faint green fluorescence which becomes rose-red on the addition of alcohol. The spectrum of this solution shows a sharp line in the green between E and b, while if the solution be quite concentrated a second, weaker line will be seen in the blue at F.

LACTOSURIA.

Milk sugar occurs occasionally in the urine of nursing women, particularly toward the end of lactation. It will reduce Fehling's and Nylander's solutions, but returns a negative result with the phenylhydrazin test. It ferments very slowly.

MALTOSURIA.

Maltose has been found in the urine of diabetic patients, and when present reduces copper and ferments with yeast. Its occurrence is sufficiently rare to be disregarded in the clinical laboratory.

PENTOSURIA.

Diabetic and morphine habitues occasionally show in the urine traces of pentose. This will reduce copper, but will not ferment.

Newmann's Orcin Test.—This test may be employed to distinguish between the pentoses, the glycuronates, and dextrose.

TECHNIC.—Three cubic centimeters of urine are treated with 10 drops of a 5 per cent. alcoholic orcin solution and 10 cubic centimeters of glacial acetic acid. The mixture is now brought to the boiling point and allowed to cool. After cooling concentrated sulphuric acid is added, drop by drop, and shaken until about 20 drops have been added. In the presence of pentoses the color becomes olive-green, glycuronates turn the solution violet, and dextrose gives a carmine color.

ACETONE.

Usually in advanced diabetics, sometimes in fever or in perfect health, particularly in patients whose diet is rich in proteids, the urine may have an ethereal odor, and will give positive reactions to tests for acetone.

Acetone is more constantly present in diphtheria and scarlet fever than in typhoid fever, and, according to recent authorities, it is the result of the reduction of food intake.

In the course of a case of diabetes a decline in the percentage of sugar, accompanied by lowered specific gravity, but without corresponding improvement in the general symptoms, may

indicate impending coma. At this time acetone will be found in the urine in increasing amounts, with or without diacetic acid and oxybutyric acid.

Ethylene-Diamin-Hydrate Test.—This is the simplest and is probably the most satisfactory for clinical purposes.

Test.—Place in a clean test-tube about 5 cubic centimeters of distilled water, and to this add about one grain of sodium nitro-prusside; shake to effect solution. Add to this an equal amount of suspected urine and thoroughly mix. Gently overlay the mixture with a few drops of a 10 per cent. solution of ethylene-diamin-hydrate. A pink or ruby-red color at the zone of contact denotes the presence of acetone. A faint white cloud, which appears on the addition of the reagent, does not denote acetone. The solution of sodium nitro-prusside must be freshly prepared.

Legal's Test.—To 5 cubic centimeters of urine in a test-tube, add a few drops of a freshly prepared solution of sodium nitro-prusside and a few drops of sodium hydrate solution. A red color will appear in all urine, which soon changes to yellow. If this is now overlaid with strong acetic acid a change in color at the line of contact from yellow to carmine or purplish red indicates acetone.

Frommer's Test may be applied to the urine direct. This test is described by Dr. Jacob Rosenbloom[7] as follows:—

"About 10 cubic centimeters of urine are treated with about 1 gram of sodium hydroxide in substance, and, without waiting for it to dissolve, 10 to 12 drops of a 10 per cent. solution of salicyl aldehyde in absolute alcohol are added. The mixture is heated to 70° C. In the presence of acetone a marked purple-red color develops at the zone of contact with the alkali. Frommer asserts that this test can indicate the presence of 0.000001 gram acetone in 8 cubic centimeters of water."

The urine should be diluted so that its specific gravity is reduced to about 1010; otherwise confusing colors occur that render interpretation difficult. When properly diluted, however, urine containing only minute or normal quantities of acetone will give after ten or fifteen minutes' standing only a straw or faintly pink color.

[7] Jour. A. M. A., August 10, 1912.

TESTS REQUIRING DISTILLED URINE.

Lieben's Test.—To a few cubic centimeters of the distillate of urine are added a few drops of concentrated sodium or potassium hydrate and a few drops of a solution of iodin in potassium iodid (Lugol's). On slightly warming the mixture yellow crystals of iodoform will separate, which may be recognized by their characteristic odor as well as by their hexagonal shape when examined under the microscope. This reaction is given by alcohol as well as by acetone but occurs more slowly. It will show amounts of acetone varying between 0.01 and 0.001 milligram per cubic centimeter.

Gunning's Test.—This is a modification of the Lieben test and is much more specific, reacting only with acetone. To the distillate from the urine are added a few drops of an alcoholic solution of iodin and the mixture treated with ammonia until a black precipitate of nitrogen iodid forms. On allowing the tube to stand for periods varying between twelve and twenty-four hours, this black precipitate disappears, leaving a yellow sediment of iodoform, which may be recognized as mentioned above. This test is less delicate than the original one of Lieben, detecting acetone when present in amounts of 0.01 milligram per cubic centimeter or more.

Test for Aceto-acetic Acid.—W. H. Hurtley offers the following new test as being the most suitable to get an idea of the amount of aceto-acetic acid in a urine. To 10 cubic centimeters of urine add 2.5 cubic centimeters of concentrated hydrochloric acid and 1 cubic centimeter of a 1 per cent. solution of sodium nitrate; shake and allow the mixture to stand for two minutes. Add 15 cubic centimeters of strong ammonia, followed by 5 cubic centimeters of a 10 per cent. solution of ferrous sulphate or a ferrous chloride solution of equivalent iron content (2 grams iron in 100 cubic centimeters); shake; pour into a Nessler glass, and allow to stand undisturbed. Do not filter. A positive reaction is present when a beautiful violet or purple color is produced. The reaction is slow, the speed with which the color develops depending on the concentration of the aceto-acetic acid present. Acetone does not give this test. The test is delicate to the detection of 1 part of aceto-acetic acid in 50,000 parts of solution.

DIACETIC ACID.

Diacetic acid does not occur in the urine of healthy individuals upon an ordinary diet, except possibly in very small quantities. It has been observed pathologically, usually in combination with ammonia, in severe forms of diabetes, in fevers, in metabolic disturbances and their coincident autointoxications, in gastric carcinoma, and in alcoholism.

Since acetone is derived from diacetic acid and the conditions requisite for the formation of diacetic acid are identical with those for the formation of acetone, the two substances are almost always found in conjunction. If little diacetic acid is formed it is all transformed into acetone; if much is formed then both substances will be found in the urine.

Gerhardt's Diacetic Ferric Chlorid Reaction.—According to the customary directions in the text-books for the performance of the Gerhardt test for diacetic acid (aceto-acetic acid), on the addition of the ferric chlorid solution to the urine a marked and obscuring precipitate of phosphates is usually obtained. This may be avoided by the chemist's simple expedient of adding the urine drop by drop to 10 to 15 cubic centimeters of the ferric chlorid solution.[8] In this way it is also possible to get a rough idea of the amount of the aceto-acetic acid present. If this is present in quantity it will give a strong Bordeaux-red coloration with the addition of a few drops of the urine; otherwise this coloration will not be present until 1 or 2 cubic centimeters of the urine has been added. Sulphocyanides, sodium acetate, salicylic acid, antipyrin, thalin and aromatic substances may produce a similar red color. For this reason the presence of diacetic acid should be assumed only after positive results have been obtained by the two following control tests[9]:—

1. Boil the urine employed for the test and the red color should be very much fainter, because boiling gradually transforms diacetic acid into acetone. 2. The urine is acidified with sulphuric acid and then some ether is added. If this is shaken with a diluted solution of ferric chlorid the aqueous layer will turn red.

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[8] F. H. Church: Jour. A. M. A., Oct. 2, 1909.
[9] Sahli: "Diagnostic Methods," 1907.

OXYBUTYRIC ACID.

This substance cannot be readily detected by chemical means unless a long and difficult preliminary process is resorted to in order to separate it from the other urinary constituents. These methods will be found in larger works on clinical and physiologic chemistry.

BILE-PIGMENTS.

General Considerations.—Bile-pigments are never found in the urine under normal circumstances. As a rule the freshly voided urine only contains the oxidized derivative, bilirubin. If a cystitis should exist, then subsequent oxidation products, biliverdin, bilifuscin, biliprasin, and bilihumin, may also be encountered.

Bile-containing urines present a more or less characteristic appearance; their color may vary from a bright golden-yellow to a dark greenish-brown. On microscopic examination it is usual to find the morphologic elements of the urine stained yellow or reddish-brown. The same color may be imparted to the foam on shaking.

Gmelin's Test for Bile-pigments.—Put some yellow nitric acid in a test-tube and gently overlay it with the suspected urine. In the presence of bile-pigment a play of colors will be observed at the zone of contact. Green will be seen nearest the urine and orange in the upper part of the nitric acid. This test is exceedingly sensitive, indicating the presence of bile-pigment in a dilution of 1 to 80,000.

Nitric Acid-Paper Test.—Moisten a piece of white filter or blotting paper with the suspected urine, and place it on a glazed tile or slab of porcelain. Allow one or two drops of commercial nitric acid to fall into the center of the wet paper. In the presence of bile-pigment concentric rings of blue, violet, green, and yellow will appear. A slight red reaction cannot be considered positive, as it may be produced by other substances than bile-pigment.

Methylene-blue Test.—A. Torday and A. Klier,[10] in working with the stomach contents of a jaundiced patient, found

[10] Deutsche med. Wochen., August 19 and 26, 1909.

that the methylene-blue with which they were testing gave a green color instead of the usual blue. The urine of a jaundiced patient gave the same reaction. Investigating further, they found that various staining fluids gave similar reactions with the bile-stained urine. Pure bile-pigments have not been studied. The method consists of adding 1 drop of a 1 per cent. solution of the dye to 15 cubic centimeters of water, and to this 1 cubic centimeter of urine. Methylene-blue gives a green color; other stains give different reactions. Diazo positive urines also turn green with methylene-blue, but a much stronger solution of the stain is required than for bile. The delicacy of this test was found to be about twice as great as the iodine or the Gmelin test.

Smith's Test.—This test has been described under other names, as those of Trosseau, Kathrein, Rosin, and Marechalt. A few cubic centimeters of urine, acidified if necessary with acetic acid, are treated with a 1 per cent. alcoholic solution of iodine in such a way that the latter solution is superimposed upon the urine, forming a distinct line of contact. If bilirubin or other biliary pigments be present, a beautiful emerald-green color is observed at the point of contact. This test is not very sensitive, indicating only 1 part of biliary pigment in 10,000 of urine. Certain drugs, especially antipyrin, may lead to the formation of a green color with this test. Thymol, if used as a preservative, may give rise to confusion with this and other bile tests.

Rosenbach's Test.—This is a modification of Gmelin's test and is performed as follows: A large quantity of urine which has been acidified with HCl is filtered several times through a thick filter-paper, which will hold back the bile-stained elements of the urine. It is sometimes advisable to add a little milk of lime to the urine before filtering, instead of the HCl, as this will throw down the phosphates, which will carry with them the biliary pigments. If the filter-paper and contents be dried by pressing with a second dry filter-paper and a drop of yellow nitric acid allowed to fall upon it, distinct rings will be seen, which will be colored as in the nitric acid-paper test, the green one being external.

Nakayama's Test.—This is a modification of the older Huppert test. Five cubic centimeters of acid urine are treated

with an equal volume of 10 per cent. barium chlorid solution and the mixture centrifuged. The barium chlorid precipitates the phosphates and sulphates and carries down the biliary pigments. The supernatant fluid is then poured off and 2 cubic centimeters of the following reagent are added to the precipitate. The reagent consists of 99 cubic centimeters of 95 per cent. alcohol, 1 cubic centimeter of concentrated HCl, and 0.4 gram of ferric chlorid. If this mixture of precipitate and reagent be heated to boiling, a bluish-green or a brilliant-green solution is obtained, which becomes violet or red on the addition of nitric acid. This test is said to indicate 1 part of bilirubin in 1,200,000 parts of urine. This test is more complicated than Gmelin's and requires more time; yet the beautiful results obtained by it more than compensate for this difficulty.

Import.—When bile is not freely and normally discharged from the bile-passages, the coloring matter from the retained bile is absorbed by the lymphatics, the various body tissues become stained with it, and it is partly eliminated in the urine.

BILE ACIDS.

Hay's Test.—This test depends upon the reduction of the surface tension of the urine in the presence of the bile acids. As advocated by Beddard and Pembrey, a pinch of powdered sulphur is sprinkled upon the surface of urine, which should be preferably at a temperature not over 17° C. In normal urines the sulphur will float upon the surface, while if the urine contains bile acids the sulphur may sink at once, indicating 1 part in 10,000, or may sink only after a few seconds to one minute, thus indicating 1 part in 50,000.[11]

Oliver's Test.—This test is based upon the well-known property possessed by the bile acids of precipitating peptone when in acid solution. (For reagent see Appendix, page 407.)

One to 2 cubic centimeters of clear filtered urine are placed in a test-tube and treated with 5 cubic centimeters of the reagent. In the presence of bile acids a decided milkiness appears at once, being the more intense, the larger the amount of bile acids.

[11] According to Sahli, this test does not discriminate between biliary acids and biliary pigments, but clinically it is a matter of indifference which are present. Phenol or aniline compounds lower the surface tension of the urine, so that their presence may lead to wrong conclusions.

Pettenkofer's Test.—To the solution of the bile-salts (see foot-note) add, very slowly, two thirds of its volume of concentrated sulphuric acid, so that the mixture does not become overheated. Then add 3 to 5 drops of a solution of 1 part of cane sugar in 4 to 5 parts of water, and shake, whereupon the liquid turns a beautiful violet color.

UROBILIN.

General Considerations.—Urobilin is closely allied to the normal urochrome, but is an abnormal product, probably resulting from the action of reducing agents upon the normal urinary pigment. It is said to be identical with the stercobilin of the feces, and its behavior to chemical reagents can be tested by testing an alcoholic extract of feces.

Test for Urobilin.—(a) Mix some urine in a test-tube with an equal amount of a 10 per cent. solution of zinc acetate. Filter off the precipitate, and the filtrate, in the presence of a demonstrable quantity of urobilin, will show a beautiful greenish fluorescence. With the polariscope the urobilin in this solution gives a distinct absorption spectrum.

(b) Fill a clean test-tube three quarters full of urine, and add one drop of strong hydrochloric acid; to this add about one-sixth volume of amylic alcohol, and after shaking slowly eight or ten times allow to separate by standing. Pour off the supernatant fluid and add to it thrice its volume of alcohol. Prepare separately a 5 per cent. solution of zinc chlorid, and add one drop of this to the alcoholic extract of the urine; finally add one drop of ammonium hydroxid. Zinc hydrate will be precipitated, which should be filtered off. In the presence of urobilin the filtrate will present a beautiful green fluorescence.

Significance.—Urobilin has been detected in cases of hepatic cirrhosis, malarial anemia, carcinoma, Addison's disease, and pancreatic disease with acholic stools. The estimation of urobilin gives a valuable indication as to the amount of blood being

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NOTE.—Hoppe-Seyler (Handbuch der Physiol. u. path.-chemischen Analyse, 1893, p. 378) gives the following directions for separation of bile-salts: Lead acetate and a very little ammonia are added to the urine. The resulting precipitate is washed with water, then boiled with alcohol and filtered while hot. A few drops of 10 per cent. sodium hydrate are added to this solution, which is then evaporated over a water bath till dry, and the residue boiled out with absolute alcohol, in which the sodium salts of the bile-acids dissolve. The filtered alcoholic extract is evaporated to a small volume, when the resinous precipitate may be dissolved in a little water, and Pettenkofer's test applied.

broken down within the body, and, therefore, serves as an early indication of the failure of the liver to functionate normally.

HEMATURIA.

General Considerations.—Blood may gain access to the urinary tract in many ways, and appear in the urine in varying amounts, from the smallest trace, demonstrable only by chemical means, to sufficient to produce bloody urine with clots.

Blood from the kidney is usually well mixed with the urine, to which it imparts a brownish, smoky hue. Under the microscope tube-casts of blood-cells may be found.

Blood from the ureter may be well mixed with the urine, or may appear in characteristic worm-like clots.

Blood from the bladder may, or may not, show irregular clots. When recently shed it imparts a bright-red color to the urine; it is frequently accompanied by much mucus and large, flat epithelial cells in great numbers.

Blood from the prostate, examined by the three-glass test, appears in the first and third glasses only.

Blood from the urethra appears in the first glass, and is frequently clotted.

Urine may show the presence of blood in the absence of any demonstrable lesion of the genito-urinary tract. It has been noted after the ingestion of strawberries, gooseberries, or a large amount of rhubarb.

The blood may be contaminated from menstrual discharge. This possibility should always be borne in mind, and false conclusions guarded against.

Microscopic Appearance.—The most convenient method of demonstrating blood in the urine is by microscopic examination of the centrifugated or sedimentated sample. The corpuscles in ordinary acid urine maintain their characteristic bi-concave shape for a number of days, if decomposition and putrefaction are prevented. If the urine is of high specific gravity, the cells rapidly become crenated. On the other hand, if the urine be alkaline or becomes so after voiding, the corpuscles will appear swollen, shriveled, or shadowy. Urine containing more than a trace of blood is albuminous.

Test for Occult Blood in the Urine.—To 10 cubic centimeters of urine in a large test-tube, add about twice as much ether and agitate thoroughly by pouring from one test-tube to another several times. To this add a few grains of powdered gum guaiac, and again agitate. Next add 5 cubic centimeters of glacial acetic acid (99.4 per cent.) and again agitate; allow this to settle and then pour off the supernatant liquid and divide equally between two test-tubes; set one aside for a control, and to the other add about 2 cubic centimeters of fresh hydrogen dioxid from a pipette, making an effort to have it settle to the bottom as a distinct layer. If a bluish discoloration appears either at the zone of contact or throughout the mixture, the presence of blood is demonstrated.

A Simple Test for Blood in the Urine.[12]—About 10 cubic centimeters of urine are filtered. Before the last few drops of urine have left the filter, a little acetic acid is added and then a mixture of tincture of guaiac and ozonized turpentine poured on. A blue color appears almost at once, if blood be present. This modification is said to be far more sensitive than the original guaiac-turpentine method, even if the latter is used after extraction with ether. It is only in dilutions as high as 1 drop of blood to 10 liters of urine that the test fails. Practically the only source of error is the presence of pus in the urine. The latter, however, does not interfere if the urine be boiled for a moment and cooled before subjecting it to the test.

HEMOGLOBINURIA.

This term signifies the presence of hemoglobin in the urine free from blood-corpuscles. Besides the foregoing test it may be demonstrated by means of the spectroscope. Faintly acid urine containing traces of hemoglobin will give two characteristic absorption bands of oxyhemoglobin. By the addition of a minute quantity of ammonium sulphid, the spectrum is changed to that of reduced hemoglobin.

Hemoglobinuria occurs in a variety of conditions: scurvy, pyemia, purpura, typhus fever, poisoning from arsenic, phosphorus, carbolic acid, chloral, and potassium chlorate. There has also been noted a periodic form of obscure origin.

[12] Wackers, Münch. med. Wochen., 1911, No. 4.

PYURIA.

Pus, being an albuminous fluid, will cause urine containing more than a minute quantity to respond to the tests for albumin. Urine containing much pus is turbid, and deposits on standing a white or greenish-white sediment which is insoluble in heat and in dilute mineral acids. The addition of hydrogen dioxid produces rapid effervescence.

The sudden appearance of pus sediment in the urine suggests the rupture of an abscess into the urinary tract, provided that the other clinical signs correspond with this assumption. The occurrence of thread-like formations is very characteristic of gonorrhea and gonorrheal sediment. They consist of pus cells glued together with mucus.

In the females the pus sediment may come from the vagina. To exclude such a possibility, either the vagina must be thoroughly irrigated before the urine is voided or the urine must be drawn with a catheter.

With alkaline fermentation a pus sediment is oftentimes converted into a ropy, gelatinous mass by the swelling of the pus corpuscles.

Microscopic Examination.—The pus corpuscles voided in the urine vary in their microscopic appearance, which depends partly upon the length of time since their escape from the blood-current, and partly upon the consistence of the urine, or upon the nature of the affection in question. Sometimes they are very cloudy and shrunken, so that the nuclei can be seen only after the addition of acetic acid (usually polynuclear or with nuclei irregularly crumpled); sometimes in an alkaline urine they are much swollen and glossy, and in this case also the nuclei are not easily seen.

Donne's Test for Purulent Sediment.—To the suspected sediment add a small piece of caustic potash, and stir with a glass rod. Pus will become thick, tough, and gelatinous, while mucus will become flaky and thin.

Significance of True Pyuria.—The presence of pus in the urine indicates the presence of an inflammatory process in the genito-urinary tract the location of which can, in some measure, be determined by the character of the associated epithelial cells (see page 290).

EPITHELIA

The epithelial cells found in urine may come from any part of the genito-urinary tract. Their forms vary greatly, and with a knowledge of the characteristic cells belonging to the different regions, it is possible, with more or less certainty, to determine their origin by their appearance. The puss cell may be taken as the standard of size.

Cells from the tubules of the kidney are round and about one-third larger than a pus cell.

From the pelvis of the kidney, twice the size of a pus cell and cuboidal or pear-shaped.

From the ureter, round and slightly smaller than from the pelvis.

From the bladder, flat and square. These are the largest cells encountered, except those from the vagina.

From the urethra, smaller than from the bladder; they may be cuboidal or columnar. All epithelial cells are granular and contain a relatively small nucleus.

TUBE CASTS.

General Considerations.—In the presence of albuminuria or hematuria, microscopic examination of the urinary sediment will, as a rule, reveal the presence of tube casts. Occasionally some varieties of casts will be found in urine which show neither albumin or blood.

The Sediment.—In order to obtain a sediment for microscopic examination, some method of precipitating and of concentrating this precipitate is necessary for a conclusive examination. A centrifuge is the most rapid as well as the safest method to employ. This process only requires from 15 to 20 cubic centimeters of urine, and can be accomplished in a few minutes. After centrifugation a part of the supernatant urine is poured off and then, with the aid of a pipette with a small point, a drop or two is drawn up and transferred to a clean microscope slide for examination.

The method of employing a conical glass may be used if a centrifuge is not at hand. Sedimentation by this means requires a number of hours, and, if care is not taken to prevent

bacterial contamination and if the specimen is not kept in a cool place, the specimen may decompose and the morphologic elements be destroyed.

The sedimentation glass is valuable for roughly estimating the amount of gross sedimentation in phosphaturia, pyuria, etc. (Fig. 50.)



FIG. 48.—"LARGE WHITE KIDNEY." × 350.

h, Hyaline cast. *g*, Spiral cast. *w*, Waxy cast. *f*, Fat-granule cast with: *n*, fat needles. Still finer needles of this type upon the neighboring fat-granule spherule. *k*, Fat-granule cell. *l*, Leukocyte. *s*, Vaginal epithelium. *t*, Fat-droplets.



FIG. 49.—CHRONIC BRIGHT'S DISEASE (CHRONIC PARENCHYMATOUS AND INTERSTITIAL NEPHRITIS). × 350.

h, *g*, *e*, *w*, Hyaline, granular, ephithelial, and waxy casts. *ep*, Renal epithelium. *vep*, Quite uniformly fatty renal epithelium.

Varieties of Casts.—HYALINE CASTS are almost transparent and appear as ground glass. They have a delicate but definite outline, and are quite friable.

Strong illumination of the field may obscure them entirely, as they are very delicate in outline and structure. They are best seen with the plane side of the reflector, and side illumination which should not be too bright.

The particular significance of hyaline casts is not yet posi-

tively settled. They occur in advanced grades of nephritis and again in transient albuminurias, and even in the absence of demonstrable albumin; they are frequently seen during the course of fevers, particularly typhoid fever.

Granular Casts.—These are more opaque than the hyaline, and are, therefore, more easily seen. The granules are numerous, and upon close examination will be found to permeate the matrix of which the cast is partly composed. This will serve to differentiate them from the pseudo-granular casts, which are merely hyaline casts to which granular *débris* has become attached during centrifugation or sedimentation. These latter



Fig. 50.—Sedimentation Glasses.

A Clear. B, Slightly cloudy with beginning precipitation. C, Sedimentation complete.

are of no more significance than hyaline casts. Granular casts are probably composed of a hyaline matrix which has undergone degeneration. They frequently show fragmented cells and fat globules in their structure. The continued occurrence of hyaline and granular casts in the presence of a permanent albuminuria, usually denotes chronic interstitial nephritis.

Hyalo-granular Casts. — Hyaline casts are occasionally found, parts of which are distinctly granular throughout their substance. They apparently represent a stage of cast-formation between the hyaline and granular varieties, and their significance is probably the same as hyaline casts.

Epithelial Casts. — These casts are composed of renal

epithelial cells (slightly larger than pus cells), grouped in the form of a short cylinder and cemented together with a hyaline or mucoid matrix. The cells may be either whole or fragmented, clear, opaque, or granular. They are significant of an acute desquamative process, resulting from renal inflammation.

Fatty Casts.—These casts may possess any of the characteristics of the preceding varieties, and present in addition free fat-globules scattered throughout the cast. They are considered as proof of fatty degeneration of the kidney.

Blood Casts.—These casts are composed either of coagulated blood, in which innumerable corpuscles in various stages of disintegration are embedded, or they may represent a hyaline matrix in which appears a varying number of red blood-cells. Some hyaline casts, which show a few blood-cells adherent upon their surfaces, may be simply hyaline or granular, to which the red cells have become attached in the bladder or after voiding. The presence of true blood casts indicates hematuria of renal origin.

Bacterial Casts.—These resemble the granular variety, except that they are more closely and more uniformly granular. The bacteria may be so numerous that the cast is almost opaque. They denote the occurrence of an acute bacterial infection of the kidney, and are rarely found.

Waxy Casts.—These are of rare occurrence, and are probably simply a dense variety of the hyaline cast.

Crystalline Casts.—As the name implies, they are crystalline in nature, being composed usually of uric acid, and more rarely of oxalates. They are very rarely encountered.

CYLINDROIDS.

These are narrow, ribbon-like bands which usually present longitudinal striations, which may or may not extend throughout the entire length of the cylindroid.

They are essentially hyaline in nature, and are probably formed from a hyaline basis. They are of renal origin, and are encountered in about 75 per cent. of cases which ordinarily come to the physician in the course of practice.

Their significance is slight, though they are considered to

indicate a degree of kidney irritation. In this connection it is of interest to note that when found they are many times accompanied by a high specific gravity, and oxaluria, with or without the presence of indican.

Microscopic Appearance.—Cylindroids appear as faint, ribbon-like bands, seen best by low illumination, and frequently exceeding in length the diameter of the field of the microscope when viewed through an objective of moderate power. Longitudinal striations may usually be detected, often running through only a portion of their length, the ends of which, after making one or more graceful curves, terminate in a gradual taper.

Cylindruria with Albuminuria.—This condition may occur as a transitory phenomenon after such violent exercises as bicycle racing, foot-ball or rowing; also in chronic constipation or after the ingestion of moderate or large amounts of alcohol, particularly by those unused to it. Continued ingestion of the salicylates has been found to produce it. It is often present during attacks of intestinal indigestion associated with oxaluria.

THE QUANTITATIVE DETERMINATION OF CYLINDROIDS AND HYALINE CASTS, WITH SPECIAL REFERENCE TO LIFE-INSURANCE EXAMINATIONS.

It often becomes necessary to record the relative number of casts in a given specimen, especially in reports of life-insurance examinations by examiners in the field, whose records must be interpreted by the medical director in the home office.

For this rough quantitative determining a fresh specimen is shaken to prevent sedimentation; then exactly 15 cubic centimeters are centrifuged at the usual rate for exactly three minutes. From the sediment a large smear is made and covered with a cover-glass.

Significance.—According to studies by Wm. Muhlberg, who suggested this technic,[13] from the standpoint of large insurance companies, the following valuation can be given to the findings. One or two hyaline casts are not considered of any significance, provided the urine has not a low specific gravity. Three to 5

[13] Medical Record, vol. lxxxviii, No. 26.

hyaline casts call for another specimen for examination, and if the same number of casts is again found, the risk is rejected. More than 5 hyaline casts in a single specimen of 15 cubic centimeters of urine calls for rejection of the risk.

Epithelia.—Various forms of epithelia are found in nearly all specimens of urine, and careful note should be made of their form and size, as well as of the condition of their protoplasm and nuclei. They are highly refractive, and are therefore plainly visible under the microscope, their outlines standing out clear and distinct. The general character of the epithelia found corresponds to the usual three varieties found throughout the body, viz.: squamous or flat, cuboidal, and columnar or cylindrical. All epithelia are more or less granular and possess one or more nuclei, though the latter are not always visible, having been disintegrated or become obscured by granular degeneration of the protoplasm. Epithelia are subject to certain physical alterations when in the urine. By absorption of water they swell up and become more regular in outline, the small forms thus becoming spherical. The small cuboidal or columnar epithelia are most important, since they probably come from the tubules of the kidney. The larger varieties of columnar and squamous cells come from the lower portion of the genito-urinary tract, the largest cells of all being the squamous vaginal epithelia.

Heart-failure Cells in the Urine.—Epithelial cells containing brown pigment granules occurring in the sputum have long been considered of diagnostic value, their appearance, associated with the chronic passive congestion of heart disease, having led to their being commonly known as "heart-failure cells." Similar pigment granules have been noted in the kidney epithelia during congestion. Pathologists who have studied them state that the pigment is of two types: (*a*) large dark-brown or black masses found in the neighborhood of hemorrhages, and (*b*) light-yellow to brownish-yellow granules occurring in the epithelium of the tubules without association with gross hemorrhage.[14] These cells have been shown to be similar to those found in the lungs in that they are of epithelial origin, but to differ

[14] Review, Medical Record, October 23, 1909.

from the latter in that the granules are rarely composed of hemosiderin. While the presence of such granules in the kidneys has been up to the present of little more than academic interest, an article by Bittorf[15] holds out the hope that they may prove to be of actual clinical and diagnostic importance. This hope is based upon this author having found such cells fairly constantly in the urine in cases of passive congestion of the kidneys as a result of heart disease. The cells found in the urine are of the type containing light-yellow granules. They are seen as more or less swollen, polymorphous, but usually polygonal cells of various, often large sizes, with indistinct, large, round nuclei. There may be several together or they may be attached to casts.

Significance.—Their occurrence in the urine associated with casts and a few scattered red blood-cells is an objective evidence of a circulatory affection of the kidneys, especially if the common sources of kidney hemorrhage, as tumors, infarcts, and acute nephritis, can be eliminated.

Pus and Blood Cells.—For the satisfactory study of these small elements, a higher power objective than that used for the study of crystals and epithelia is required. Oblique light, with moderate illumination, will be found best. Occasionally great difficulty is experienced in differentiating blood, pus, and small, round epithelial cells. This confusion is not so likely to occur when all varieties are present in sufficient quantity in one specimen to admit of careful comparison; but when only a few scattered cells of one variety are found, mistaken identity is quite possible. It is well, as a general guide, to bear in mind the relative size of these different elements. Pus-corpuscles are the most common, and these should be thought of first. They are identified as small granular discs, having multi-nuclei. Somewhat smaller in size (about one-third) will be noted the pale, non-granular, non-nucleated discs which are the red blood-cells. While at least one-third larger than the pus-cells, the so-called renal epithelia present a granular protoplasm with large nuclei.

Finally, after determining the individual elements in a given specimen, it is important to note the degree of degenera-

[15] Münch. med. Wochen., August 31, 1909.

tion and the presence of fatty change, particularly occurring in the casts; and, lastly, the number of each formed element per field or per drop should be determined, this observation being important in following the course of any pathologic condition, as it is similar to the repeated quantitative determination of albumin or sugar.

Bacteriuria.—The presence of excessive numbers of microorganisms, their motility, and even some of their morphologic characteristics may be determined during the microscopic examination of the sediment; a complete and satisfactory examination of their detail and identity cannot, however, be determined except by special bacteriologic technic, for which the reader is referred to larger works on bacteriology or urinary diagnosis.

Spermaturia.—The presence of semen in the urine will give positive reactions for albumin. It may be present in the urine after coitus, after nocturnal emissions, or in spermatorrhea.

Microscopic examination will reveal the characteristic elements which are motionless, but which resist decomposition for days.

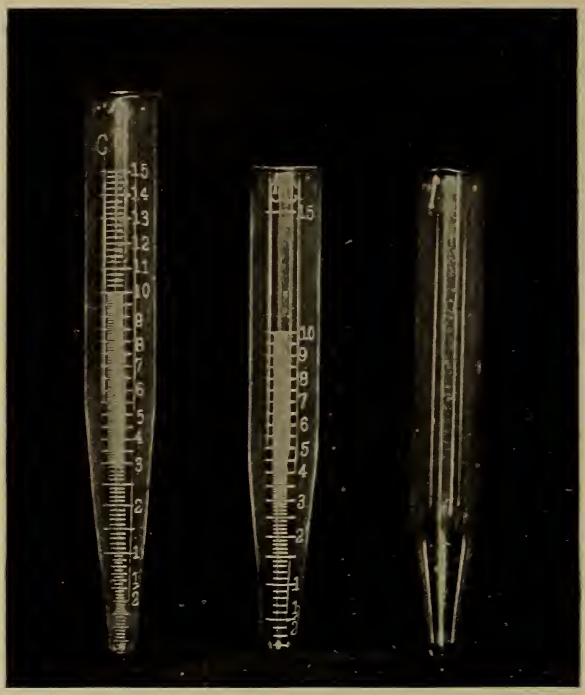
Chyluria.—Under very occasional circumstances the urine may contain chyle, which gives it the appearance of milk, and forms on standing a cream-like layer at the top. It responds to all the tests for albumin.

Microscopically, much fat is found in the form of innumerable highly refractive globules, which are soluble in ether.

A large amount of fat in the urine almost always signifies chyluria (lipuria). The urine is albuminous, of a milk-white to a cloudy-yellow appearance, sometimes even slightly blood-tinged, neutral or faintly acid, forms a cream-like layer, and often contains small coagula. The latter may form within the body as well as after the urine is voided. A microscopic examination shows that the fat is subdivided much more freely in the urine than it is in milk. No distinct fat-drops can be seen, but extremely finely divided, almost invisible, fat granules. These granules furnish the cloudiness and cream-like layers. By shaking a little of this cloudy urine with ether the fat may easily be removed, and demonstrated by evaporation of the ether residue.

THE INORGANIC SEDIMENT.

To obtain a specimen of inorganic sediment, it is preferable to centrifuge the freshly voided urine rather than to wait for the slower process of sedimentation. The use of the centrifuge precludes the possibility of the process of decomposition so altering the specimen that the original picture is destroyed.



A B C

FIG. 51.—CENTRIFUGE TUBES.

A, Correct form of graduated percentage tube (note the slender taper which facilitates reading of small per cent.). B, Improper form of graduated tube accurate reading of small percentages difficult. C, Plain centrifuge tube.

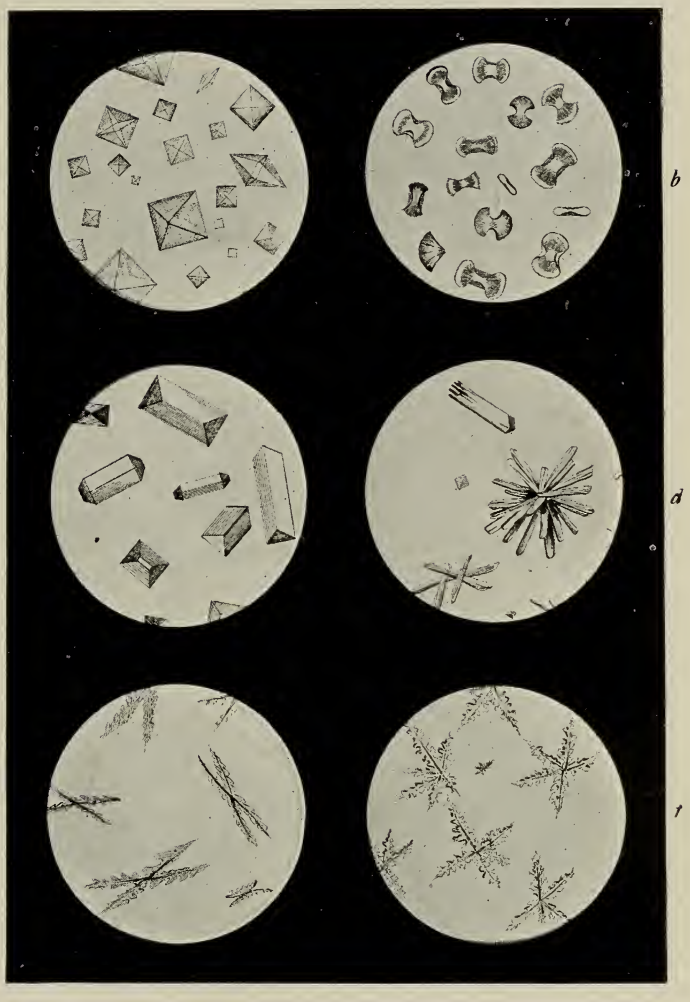
Alteration in reaction will so change the character of the crystalline deposit that it will be useless from a diagnostic standpoint. A decomposed alkaline urine will often present such a mass of phosphatic sediment that the less voluminous, but more important, elements are greatly obscured and may be overlooked.

PLATE VIII



a and *b*. Usual Forms of Uric Acid Crystals. *c*, *d*, *e* and *f*. Less Common Uric Acid Crystals.

PLATE IX



a and *b*. Calcium Oxalate Crystals. *c*, *d*, *e* and *f*. Phosphates.

PLATE X



a and *b*. Uncommon Forms of Uric Acid Crystals. *c*. Cholesterin. *d*. Cystin. *e*. Tyrosin. *f*. Leucin.

Preparation of the Slide.—The sediment should be taken from the bottom of the centrifuge tube (Fig. 51) by means of a small pipette with a long, slender tip. Not more than a drop or two should be allowed to enter the tube. This is particularly important when the amount of sediment is small. If the sediment is large and dense it should be diluted with a drop or two of distilled water and a cover-glass placed upon it. Much time will be saved by first examining the preparation with the ⅓ or ¼ objective, which is convenient for locating an interesting part of the slide, upon which the high-power objective may be focused for more careful study.

Crystalline Deposits. (See Plates VIII, IX, and X.)

Acid Group.—*Uric Acid* (Plates VIII and X): These crystals are yellow, reddish-brown or brown in color. The most characteristic forms are rhombic prisms or lozenge-shaped crystals (Plate VIII, Figs. *a* and *b*). These occur singly, but more often they are united in irregular masses. (Other more rare forms are shown in Plate VIII, Figs. *c, d, e,* and *f;* Plate X, *a* and *b.*)

Urates.—The urates, chiefly the urate of sodium and the urate of potassium, if they do not appear as an amorphous deposit, appear as crystals in the forms of needles or dumb-bells, of reddish-brown color, and also in globular masses which are dark-brown and almost opaque, with or without projecting spines.

Oxalates.—The usual form of calcium oxalate in the urine is a perfect octahedron without color. More rarely they appear in the conventional hour-glass form (Plate IX, *a* and *b*). This form is somewhat similar to the urate from which it may be distinguished by the total absence of color in the oxalates.

Carbonates.—These are rare, but when present evolve bubbles of gas when treated with hydrochloric acid under the microscope.

Sulphates.—This is a rare form of deposit which, when present, appears as fine, feathery crystals. Frequently a number of crystals radiate from a common center.

Alkaline Group.—*Phosphates:* These may occur as a semi-opaque amorphous deposit without color. More commonly they appear as the characteristic coffin-lid crystals. A less com-

mon form of crystalline phosphatic deposit (Plate IX, *c, d, e* and *f*) appears as fine, branching, feathery crystals, which have been likened to the needles and branches of the pine tree.

Ammonium Urate (see Fig. 42).—These are characteristic of the uric acid and urate group in that they are yellow or brownish in color. In alkaline urine the urates appear as fine, feathery spheres of varying size, resembling to some extent chestnut burrs.

Cholesterin.—This is a rare form of deposit which appears in the form of irregular, flat platelets whose sides follow the characteristic lines of a parallelogram, the angles of which are often irregular. Not infrequently the platelets occur in overlapping groups. (Plate X, *c.*)

Cystin.—This is a rare deposit. When present it appears as irregular transparent plates of varying size, often in overlapping groups. (Plate X, *d.*)

METHOD TO DETERMINE ROUGHLY THE NATURE OF COMMON UNORGANIZED URINARY DEPOSIT.

1. Warm the deposit and some urine. If it dissolves, the deposit is composed of urates. If it is unaffected by heat, the deposit is either phosphates, uric acid, or calcium oxalate.

2. Warm a fresh portion of urine with acetic acid. If the sediment dissolves, it is composed of phosphates; if it does not dissolve, it is uric acid or calcium oxalate.

3. Add to this undissolved portion some HCl and heat again. If the sediment now dissolves, it is calcium oxalate; if it does not dissolve, it is composed of uric acid.

URINARY CONCRETIONS.

General Considerations.—About 75 per cent. of all urinary concretions are either uric acid or urates. Next in frequency are found the calcium oxalate or mulberry concretions. More rare primary concretions may consist of blood, cystin, xanthin, calcium phosphate, or calcium carbonate.

Secondarily, any one of these formations may become covered with a whitish layer of mixed phosphates. These are pre-

cipitated upon the original concretion by ammoniacal fermentation, which occurs in the bladder, secondary to the presence of the calculus.

Analysis of the Concretion.—1. Burn a portion upon a piece of platinum foil in a Bunsen flame or blow-pipe.

A. If it chars greatly and leaves little ash, it may be uric acid, urates, cystin, xanthin or blood.

1. If it gives the murexid test, it is uric acid or urates.
2. If it dissolves in boiling water, it is urates.
3. If it does not dissolve in boiling water, it is uric acid.
4. Cystin and xanthin are very rare forms of concretions which require special tests for their detection.

B. If it chars very slightly and leaves considerable ash it may be phosphates, oxalates or calcium carbonate.

1. Treat a fresh portion with dilute HCl.
   (a) If it dissolves with effervescence, carbonates are present.
   (b) If it is soluble without effervescence, phosphates or oxalates are present. (See C, 3, below.)
2. Treat a portion with dilute acetic acid.
   (a) If it dissolves with the aid of heat, it is phosphatic.

C.—1. If it fuses to a bead on platinum foil, it is urates.

2. If it does not fuse, it is calcium phosphate.

3. If it is insoluble in either of the above acids, it is calcium oxalate.

THE DIAZO REACTION.

The Reaction.—The ammoniacal solution of the anhydride of para-diazo-benzin-sulphonic acid has the property of developing a salmon pink or red color reaction with certain pathologic urines. Normal urine never gives this reaction.

The Test (for preparations of solutions see Appendix).—Take 5 cubic centimeters of solution "A" and add three drops of solution "B," and mix together in a clean test-tube. To this

add 5 cubic centimeters of urine and again mix. Now allow a few drops of ammonia water to flow down the side of the tube. As it comes in contact with the mixture in the tube a pinkish or red color indicates a positive reaction. Upon shaking the tube the entire mixture becomes colored, and the color is also imparted to the foam. This reaction does not always appear immediately, so a few minutes should be allowed for its development before reporting a negative reaction.

Feri's Improved Diazo-reaction.[16]—Feri's modification of the test consists in the use of a single reagent, marketed under the name of "asophorroth P. N.," instead of the fresh mixture of sulphanilic acid and sodium nitrite, as recommended by Ehrlich. The method is simpler and the reagent permanent. A trace of the former is shaken up with a little water in a test-tube and a few drops of the urine, made alkaline with sodium hydrate, are added. A brilliant red color marks the positive reaction.

Significance of the Reaction.—It is not yet definitely known what particular substance or substances in the urine yield this reaction. It may occur in the presence of several aldehydes, ketones, and phenols.

A positive reaction is usually obtained with the urine of typhoid fever patients after the fourth day of the disease. Its continued absence, however, does not preclude the possibility of this disease. It is also positive in some cases of measles, pulmonary tuberculosis, etc.

Russo's Test (Methylene-blue Reaction).—This test has been recently advanced and seems to have somewhat more diagnostic importance than has the diazo-reaction. The technic is very simple and is as follows: 4 drops of a 1 to 1000 aqueous solution of methylene-blue are added to 4 or 5 cubic centimeters of suspected urine. If the reaction be positive the mixture turns to an emerald or mint-green hue. A light-green or bluish-green tint shows a negative reaction. The positive reaction is not affected by boiling the urine or by the previous ingestion of such compounds as phenacetin, salol, quinin, and calomel. The difficulty in the application of the test comes in the ability to recognize the various tints of green which may be present. With

[16] Wien. klin. Wochen., No. 24, 1912.

a little practice, however, a positive reaction may be readily detected, especially if a control test be made with normal urine.

This test is shown as early as the second day of typhoid fever and persists throughout its course. The mint-green hue is first observed, the emerald-green tint appearing as the disease progresses. If the course is favorable the color tone becomes more and more bluish, while if unfavorable the emerald tint persists. This test is also positive in measles, small-pox, chronic and suppurative tuberculosis, but it is negative in varioloid, varicella, scarlet fever, miliary tuberculosis, appendicitis, and malaria.

This test is as simple and apparently just as reliable as is the diazo-reaction, being especially valuable in differentiating a typhoid from a miliary tuberculosis.

Fallacy.—Urines containing bilirubin react to this test, so that this fallacy must be borne in mind. (See page 286.)

EXAMINATION OF URINE FOR SUBSTANCES INTRODUCED INTO THE BODY FROM WITHOUT.

(Drugs and Poisons.)

Detection of Lead.—A bright strip of magnesium, free from lead, is placed in the urine and left there for some time; the deposit which forms is dissolved in nitric acid, and then tested according to the methods of inorganic analysis.[17]

If the urine contains but a small amount of lead this method will not give the desired result; then a larger quantity of urine must be used, the organic substances destroyed with HCl and potassium chlorate, and the lead sought for in the evaporated residue.

Detection of Mercury.—To 500 to 1000 cubic centimeters of urine, add 2 to 4 cubic centimeters of HCl; then digest at 60° to 80° C., for five or ten minutes in a flask with a few bright strips of brass or copper. The metal is then washed with water, then with alcohol, and finally with ether, and dropped into an ignition tube (glass tube of high-fusing point). This is then brought to a red heat, care being exercised to keep the upper end of the tube cool. The mercury which has become amalgamated with the metal becomes volatilized, and is rede-

[17] See Marshall's "Medicus," J. B. Lippincott Co.

posited in the upper cold end of the tube, where it is seen as a bright mirror, or, if a small amount, the individual globules of mercury may be seen by a hand-lens or the low power of the microscope.

Detection of Iodin.—Iodin occurs in the urine as potassium iodid after the internal or external application of iodin or one of its combinations. It may easily be demonstrated as follows: A few cubic centimeters of urine are boiled with a piece of starch of about the size of a pea until the latter is dissolved. After cooling, the fluid is carefully overlaid with concentrated nitric acid. If iodin is present a blue-violet ring, which gradually disappears, is formed at the line of junction of the two fluids. A second method is to add to the urine a few drops of crude nitric acid and a less number of drops of chloroform, and then shake gently. If iodin is present the chloroform, which sinks to the bottom of the tube, will be colored rose-red or violet. Both the above tests are very delicate, but if the urine contains only a small trace of iodin, the chloroform test is not very conclusive, since the nitric acid may set free indol and skatol pigments, as well as urorosein, any of which will color the chloroform in a similar manner. But in this case the urine itself appears more deeply colored than the chloroform.

Detection of Bromin.—The test for bromin is performed in exactly the same manner as iodin test, except that a few drops of a calcium chlorid solution and hydrochloric acid are used to liberate the bromin. If bromin is present the chloroform will be colored yellowish-brown. This test, though far less delicate than the iodin-test, is sufficiently accurate to recognize the therapeutic ingestion of large doses of bromin salts, and is chiefly of interest in verifying the diagnosis of suspected bromism.

This method may be uncertain because of the discoloration of the chloroform by urinary derivatives. To prevent this source of error 16 cubic centimeters of urine, to which have been added 2 cubic centimeters of caustic potash and 2 cubic centimeters of potassium nitrate, are evaporated and incinerated, the ash dissolved in water, and the resulting solution tested for the presence of bromin, as above.

Detection of Salicylic Acid.—Dilute ferric chlorid is added to the urine drop by drop. If the latter becomes a more or less intense violet, the reaction is positive. Salicylic acid and its salts, in which latter form the administered salicylic acid partly appears in the urine, both give this reaction.

Detection of Phenol.—Phenol appears in the urine largely as phenol sulphate. Ferric chlorid will produce a violet-blue color in the distillate from phenol urine, to which 5 per cent. sulphuric acid has been added. Phenol urine turns dark or black on exposure to the air, when sufficient time is allowed for oxidation to occur.

Detection of Antipyrin.—The urine appears dark and is dichroic, *i.e.*, in reflected light, greenish; by transmitted light, reddish. A permanent brown-red color gradually appears upon the addition of ferric chlorid solution.

Detection of Phenacetin (Acetphenetidin).—The urine is dark yellow and turns reddish-brown on the addition of ferric chlorid solution. The color gradually becomes black after prolonged standing.

Detection of Antifebrin.—The urine is extracted with chloroform, and to the extract mercuric nitrate is added. The mixture is then heated, and if a green color is produced antifebrin is present.[18]

Detection of Pyramidon.—The urine is frequently clear purplish-red in color, and deposits a sediment composed of small, red needles. If the urine is mixed with an equal volume of a 2 per cent. solution of ferric chlorid, a dark-brown, amethyst color is produced.

Detection of Tannin.—Tannin is eliminated in the urine partly as gallic acid. Urine which contains tannic and gallic acids turn a deep blue-black upon the addition of ferric chlorid solution.

Detection of Balsam of Copaiba and Sandalwood Oil.—After the administration of copaiba the urine will reduce cupric oxid (Trommer's test), but not bismuth (Nylander's test). If HCl is added to the urine drop by drop, a precipitate of resinous acids appears with a reddish or violet coloration. Also after the

[18] Yvon: Jour. de pharm. et de chemie, No. 1, 1887.

administration of sandalwood oil the urine has reducing properties, and exhibits a precipitate of resinous acid when HCl is added, but it will be of a reddish-brown color.

Detection of Santonin.—Santonin urine is of a saffron-yellow or greenish color. The addition of sodium hydrate will turn it a rose-red. If this rose pigment is shaken with amylic alcohol, it will be immediately taken up by the latter, giving it an intense and beautiful color, while at the same time the urine is decolorized.

TESTS FOR FUNCTIONAL RENAL CAPACITY.

Since the original work of Geraghty and Rowntree,[19] who first demonstrated the practicability of the phenolsulphonephthalein test, in the study of renal efficiency, subsequent studies, both by these observers and by many other clinical investigators, have repeatedly verified their results. This test has now generally superseded the earlier colorimetric tests, such as the indigocarmin, the rosanalin, etc., because of its uniform reliability, and especially because the technic is one well suited to routine clinical work, possessing no inherent or technical difficulties, eliminating all difficult or pain-producing procedures, and being easy of interpretation. For these reasons, the author intentionally omits other tests of a similar nature, referring the reader desiring more extensive information to larger works on diagnosis or urology.

The various steps of the investigation, as originally laid down by Geraghty and Rowntree, remain unchanged except for slight and unimportant modifications, the more valuable of which will be referred to below.

The test depends upon the fact that phenolsulphonephthalein when introduced into the body is excreted with extraordinary rapidity and appears in the urine normally within a few minutes after injection, while in alkaline solution a brilliant red color is produced which is ideally adapted for quantitative colorimetric estimations.

Technic of Geraghty and Rowntree.—Direct the patient to take 300 to 400 cubic centimeters of water from twenty minutes

[19] Jour. Pharm. and Exp. Therap., July, 1910, i, 579.

to a half-hour before administering the drug. This insures free urinary secretion and prevents delayed time of appearance due to lack of secretion. The employment of a catheter to facilitate collection of the urine, as at first advocated, has largely been discarded, because of the uniformity in the time of the appearance of the drug after its administration. This has been found to be fifteen minutes, so that, in all examinations, fifteen minutes are allowed to pass before counting the first hour of secretion. Neither is it necessary to commence with an empty bladder, as the quantitative estimation depends solely on the quantity of the drug excreted, and not on the volume of urine, which may be 50 or 500 cubic centimeters without affecting the result.

Only in cases of urinary obstruction and for estimating separately the function of each kidney will catheterization have to be resorted to.

One cubic centimeter of a solution containing 6 milligrams of the drug is injected intramuscularly (it may be given subcutaneously or intravenously, if desired). The urine is first collected at the end of one hour and fifteen minutes, again an hour later, and it is usually advisable to collect a third specimen at the end of the next hour.

Sufficient NaOH (10 per cent.) is added to the collected urine to make it decidedly alkaline, as the color displayed in acid urine is yellow or orange, and may not be observed. The collected solution is placed in a 1-liter measuring flask and diluted with distilled water to the 1-liter mark. This solution is thoroughly mixed and then a small filtered portion used to compare with the standard tubes used in all these estimations.

Probably the best instrument for the tint comparison is the Duboscq colorimeter. It has the disadvantage of being expensive. It is advisable to make a comparison soon after voiding, and, if this is not possible, to render the urine distinctly acid, as the presence of alkali causes the red color to gradually fade in a few hours.

Additional Technic.—In the presence of marked urinary obstruction, or retention, a catheter should be introduced and allowed to remain until the end of the third period of collection, three hours and fifteen minutes. To investigate the excretory power of each kidney separately, the catheterizing cystoscope

will have to be employed, and the urine from each kidney collected and examined separately.

In instances in which the pigment elimination is so slight as to be practically lost by the usual dilution up to 1 liter, the voided or catheterized specimen, after being alkalinized with 20 cubic centimeters of a 10 per cent. sodium hydrate solution, which is necessary in every instance, in order to bring out the color value, the dilution should be made only to 250 or 500 cubic centimeters and allowance made for the fractional dilution when comparing with the colorimeter.[20]

Significance of Findings.—Normally, the largest amount is eliminated at the end of the first hour and fifteen minutes, the amount varying from 30 to 50 per cent., with 15 to 25 per cent. at the end of the next hour, and merely a trace in the third specimen. Abnormally this condition is reversed, and the greatest amount is eliminated in the second or even the third hour, and in uremia or impending uremia elimination is often too slight to permit of definite reading in any of the specimens (Robertson).

Following *intravenous* injections the drug appears in three to five minutes; 35 to 45 per cent. is eliminated in the first fifteen minutes; 50 to 65 per cent. in the first half-hour and 63 to 80 per cent. during the first hour. For general use the lumbar intramuscular method is advocated especially when edema is present (Geraghty and Rowntree).

Effect of Drugs.—The output does not seem to be influenced by the previous administration of the different diuretics, as caffeine, urea, calomel, etc., whereas those affecting the blood-pressure and osmotic tension, as the nitrites, and digitalis may slightly decrease the phthalein output.

Value in Surgery.—Tests of 100 cases show it to be a valuable guide as to the ability of the patient to stand anesthesia during operation.

Application of test to determine functional activity of individual kidneys is useful in cases of unilateral and bilateral disease; here the urethral catheter must be used. Under these conditions the time of injection is recorded, and also the time of appearance of the drug on each side. Starting from the time of

[20] W. E. Robertson: N. Y. Med. Jour., May 16, 1914.

the appearance, the collection is then continued for one hour. The amount of drug in each specimen is then estimated as above.

Results in Normal Cases.—The time of appearance on two sides is usually simultaneous. The amount normally excreted of each kidney will be practically the same. Under surgical conditions, the test is of great value in demonstrating the doubtful diseased condition of one kidney and to prove the functional capacity of the other kidney in cases where operation on the kidney is indicated.

It is of immense value from a diagnostic and prognostic standpoint in nephritis, inasmuch as it reveals the degree of functional derangement in nephritis, whether of the acute or chronic variety.

In the cardiorenal cases the test may prove of value in determining to what degree renal insufficiency is responsible for the clinical picture presented.

The test has proven of value not only in diagnosing uremia from conditions simulating it, but has also successfully indicated that uremia was impending when there was no clinical evidence of its existence at the time.

The test has proven of great value in revealing the true renal condition in cases of urinary obstruction. It is here of more value than the urinary output of total solids, urea, or total nitrogen, and enables the surgeon to select a time for operation when the kidneys are in their most favorable functional condition. The improvement in the renal condition in cases of urinary obstruction following the institution of preliminary drainage is strikingly indicated by this test.

To obtain the most accurate results recourse must be had to other tests related to renal functional capacity. A complete examination should include, besides a repeated phenolsulphonephthalein test, an indigo-carmin test, by means of the cystoscope, and an estimation of twenty-four hours' nitrogen elimination with the diet under control as far as the nitrogenous intake is concerned.

URINARY TESTS FOR FUNCTIONAL ACTIVITY OF OTHER ORGANS.

Cammidge Reaction.[21]

This reaction is based upon the presence of certain, at present unknown, substances, which occur in the urine in pancreatic disease associated with fat necrosis, the detection of which is based upon some special reactions in which phenylhydrazin hydrochlorid plays an active part.

Many observers have followed the methods of Cammidge in the determination of this reaction and its relation to disease of the pancreas. Cammidge himself, realizing the non-specificity of the original A- and B-reactions, later added a third or C-reaction, in an effort to render the findings more conclusive. In spite of this we are as yet unable to place any great confidence in the findings of this test, the results obtained by a number of careful and competent investigators being so at variance that it seems doubtful if this test will ever be established upon a sure and practical foundation.

The difficulty may be due in part to the inherent difficulties in the technic itself and to the extreme delicacy of the reaction. The slightest variation in the performance of its various steps often rendering valueless a large amount of tedious and time-consuming work, we must conclude, after a careful analysis of the available, clinical and experimental data pertaining to this reaction and its relation to the diagnosis of pancreatic disease that it is still in an experimental stage, and that many uncertain and negative results in undoubted lesions of the pancreas make it impossible to place a definite value upon the findings; this together with the difficulty with the test itself removes the Cammidge reaction as an available clinical procedure from daily use by the clinician.

DIMETHYLAMINOBENZALDEHYD REACTION FOR FUNCTION OF LIVER.

This test, advanced by Ehrlich, is as follows: Prepare a 2 per cent. solution of p-dimethylaminobenzaldehyd in equal parts

[21] See "Diseases of Pancreas," Robson and Cammidge, 1907, for further information. Also Schumm and Heyler: Münch. med. Wochen., Feb. 14, 1909; Jour. A. M. A., Aug. 22, 1908; J. E. Schmidt: Mitteilungen aus den Grenzgebieten der Med. und Chir., Jena, July 24, 1909; Speese and Goodman: N. Y. Med. Jour., Aug. 14, 1909; Cammidge: Lancet, March 19, 1904; Mayo Robson: Lancet, page 773, 1904.

of concentrated HCl and water. Add a few drops of this solution to 5 cubic centimeters of fresh cold urine and allow to stand for a few minutes. A positive reaction is indicated by the appearance of a cherry-red color, which may be extracted with chloroform. Heating facilitates the reaction, but here normal urine may give a slight reddish coloration. In the cold normal urine gives a greenish-yellow color.

Significance.—Apparently this coloration is due to the presence of metabolic products derived from blood-pigments (urobilinogen compounds). It is to be expected, therefore, that this reaction would be distinct in diseases of the liver and bile passages, although it is not constant even here. It is also observed in tuberculosis, pneumonia, typhoid fever, and malaria.

From his studies of this test, Allen Eustis[22] suggests the following additional steps: If urobilinogen is present a scarlet-red color appears, which persists on dilution with water. While urines in which there is no urobilinogen may give a reddish color (due to pyrrhol derivatives) on the addition of the reagent, on diluting with water the reddish tinge changes to yellow, while those urines giving a positive reaction change to pink, the color persisting up to a very high dilution.

HIROSE TEST FOR FUNCTION OF LIVER.

This observer[23] recommends the use of galactose to measure carbohydrate tolerance because the normal limit of tolerance in the fasting state is $\frac{1}{10}$ that of cane sugar (or 20 to 40 grams daily).

The test is applied after fasting, with special reference to milk, by giving 24 grams chemically pure galactose, and then examining small specimens of urine for this sugar during a period of twenty-four hours.

The test possesses no specific value, but may be found to be of value in measuring hepatic insufficiency.

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[22] New Orleans Med. and Surg. Jour., December, 1912.
[23] Deut. med. Wochen., July 25, 1912.

IX.

BODY FLUIDS, EXUDATES, TRANSUDATES, AND SECRETIONS.

CEREBROSPINAL FLUID.

General Considerations.—Since the introduction of lumbar puncture by Quincke, the cerebrospinal fluid has gained so much importance from a diagnostic standpoint that much of value may be learned from a systematic study of this fluid. There is little danger in the performance of lumbar puncture, as the spinal cord does not reach to the usual point of puncture and the fibers of the cauda equina are sufficiently movable to escape the needle. While few effects are observed in the ordinary run of cases, a few have been reported in which symptoms of collapse are evident. It should be a rule, therefore, to stop proceedings if such symptoms arise and also to keep the patient quiet in bed for at least twenty-four hours following the puncture, or until the pressure in the cerebrospinal cavities may become equalized.

The examination of the cerebrospinal fluid conveniently divides itself into three groups: (*a*) A study of the cerebrospinal pressure; (*b*) an examination of the physical characteristics of the fluid, including its general chemistry; a microscopic or cytologic study; (*c*) a study of special more or less specific reactions and a bacteriologic examination. The most valuable of the clinical tests are those proposed by Nonne and Appelt, but modified and improved by Ross and Jones,[1] and later by Noguchi and Moore.[2] This test is based upon the fact that in inflammatory conditions of the meninges the cerebrospinal fluid contains an increased amount of globulin, while in other pathologic conditions of the central nervous system,

[1] British Medical Jour., May 9, 1909.
[2] Jour. Exper. Medicine, vol. ii, p. 604, 1909.

non-inflammatory in nature, the amount of globulin is present in amounts not demonstrable by ordinary chemical methods. The original test of Nonne and Appelt devised to demonstrate this increased globulin content, and known as the Nonne "Phase 1" reaction, has given way to the modification of Ross and Jones and the Noguchi tests on account of the greater delicacy and ease of interpretation of these latter.

Physical Characteristics.—Normal cerebrospinal fluid is colorless, limpid, and practically free from morphologic elements. Its specific gravity ranges between 1002 and 1010. It is alkaline in reaction, the degree of alkalinity varying between 15 and 20. It contains a trace of protein and about 0.1 per cent. of glucose.

Pathologically the fluid may be clear or very cloudy, due to the presence of leukocytes, erythrocytes, and endothelial cells. This cellular admixture may be so extensive as to give the appearance of pure pus. In cases of cerebral hemorrhage from the ventricles, hemorrhagic pachymeningitis, or traumatic lesions of the spinal cord, enough blood may be present to give the appearance of practically pure blood, the color varying from a bright red to a brownish or greenish red, depending upon the length of time it has been shed and has remained admixed with the cerebrospinal fluid. This admixture with blood may lead to the spontaneous coagulation of the fluid, which may serve as a differentiating point between inflammatory and non-inflammatory lesions. Thus, in tuberculous meningitis very slight coagulation may be observed, while in the epidemic cerebrospinal meningitis the coagulum may be very firm.

The Chemical Examination of the cerebrospinal fluid offers some points of clinical value. While the albumin content normally is much less than 0.1 per cent., it may vary under pathologic conditions to as high as 0.8 per cent. The total protein, especially the euglobulin portion, is increased in all cases of acute inflammations of the meninges, in hydrocephalus, in syphilitic and in parasyphilitic diseases of the cerebrospinal tract. Glucose is usually present, but may entirely disappear under pathologic influences due to an autolysis which is controlled by the leukocytic ferments, the glucose being converted into lactic acid. Cholin is present normally as a mere trace,

while pathologically the amount may vary between 0.021 and 0.046 per cent.

To Obtain the Specimen.[3]—It is very important when withdrawing the cerebrospinal fluid to see that too much is not abstracted at one time. Death has resulted from too rapid and too great removal of fluid. This risk need hardly be considered in obtaining fluid for purposes of examination, as not more than 8 to 10 cubic centimeters are required. Untoward symptoms are less likely to follow spinal puncture if the patient is kept in bed for a few hours succeeding the procedure.

The control of the needle is important. It is quite possible with an 8-centimeter needle to thrust into the peritoneal cavity or between the bodies of the vertebra, and by this latter path to pierce even the vena cava. Aspiration to start the flow is not permissible, as with proper precautions and careful technic a dry tap is very rare.

The Pressure.—For exact pressure-determinations elaborate apparatus is not required. For ordinary bedside work the apparatus about to be described is sufficient: As soon as the subarachnoid space has been entered, as shown by the appearance of fluid in the needle, a thick-walled glass tube of fine bore (about 1½ millimeters diameter) is joined to the needle by a short rubber connection, and the fluid allowed to find its level in this tube held vertically, and the pressure measured in terms of millimeters according to the height to which the fluid rises. The capillary error, variations due to the differing specific gravity in the fluid, and the change due to loss of fluid from the spinal canal, may be neglected for all practical purposes. Under ordinary bedside conditions, with this apparatus, the normal variations in cerebrospinal pressure are between 50 and 300 millimeters of the fluid itself or between 5 and 7.5 millimeters Hg. By careful experimentation and elaborate apparatus, the normal variations have been found to lie between 120 and 180 millimeters of distilled water.

Physiologic Modifications.—Crying or coughing during the examination will cause a rise in pressure of 50 millimeters or more. Posture also influences pressure, as is shown if the

[3] Abstract of article by Francis Peyton Rous, M.D., in International Clinics, vol. ii, Seventeenth Series.

patient (who should be reclining horizontally on one side) raises his head. The column further shows fluctuations synchronous with the pulse and respiration. Finally the absolute pressure will be higher than normal if the patient exerts any muscular force during the examination. Muscular exertion should be eliminated as much as possible during the reading.

Pathologic Modifications.—Marked increase in pressure is the rule in hydrocephalus, in brain tumor, and in meningitis of bacterial origin, especially when the infection is acute. Increase in pressure often accompanies uremic coma. A column of 600 to 700 millimeters of fluid is not uncommon in any of these conditions. Low pressure is observed in infants in conditions giving low blood-pressure (see section on "Blood-pressure"), and when an error in technic has occurred.

Collection of the Specimen.—When the pressure has been determined, disconnect the manometer and collect a few cubic centimeters in a sterile capillary tube as the fluid emerges from the needle. This specimen should be preserved under aseptic precautions for bacteriologic examination; from 4 to 6 cubic centimeters more should be allowed to run directly into a clean centrifuge tube for cytologic examination.

CYTOLOGIC STUDIES.

Determination of the Cell-Content.—In the normal cerebrospinal fluid there are from one to seven white cells per cubic millimeter. Probably they are never entirely absent. When the meninges are sound none but lymphocytes occur; these are often much degenerated. The fluid is normally clear, and even if it contains several hundred cells per cubic millimeter, it may remain clear macroscopically.

The cells may be counted with the Thoma-Zeiss hemocytometer, using the "red" pipette for measuring. Since there is usually some hemorrhage accompanying the puncture, it is necessary to have previously counted the number of red and white cells in the patient's blood to determine the ratio of these cells in the circulation, so that after counting the red and white cells in the cerebrospinal fluid it will be possible to determine how many white cells are adventitious (due to hemorrhage). To determine the cell-content of the cerebrospinal fluid it is only

necessary to place a drop of the freshly drawn fluid upon the depression in the chamber and apply the cover-glass; to count the red cells one filling will be all that is necessary. For the white cells, if the cells are scant, five or ten complete fields must be counted and an average obtained.

Example.—Suppose in the specimen we find 80,000 red cells and 102 white cells per cubic millimeter, while in the blood itself there are 4,000,000 reds and 5000 whites, or a proportion of 800 to 1. Calculating on this ratio of the 102 white cells present per cubic millimeter of the cerebrospinal fluid, 100 are from the contamination blood. Thus, but two white cells per cubic millimeter were present in the fluid before contamination by hemorrhage.

Method of Baybee and Lorenz.—In the method of Baybee and Lorenz[4] the cells are counted in a Thoma-Zeiss blood-counting chamber, in which the spinal fluid is first mixed in the red blood-counting pipette with a diluting fluid composed as follows:—

Methyl violet	0.1	Gm.
Glacial acetic acid	2	c.c.
Aq. dest.	q. s. ad 50	c.c.

The technic is as follows: Draw the diluting fluid up into the pipette to division 7, remove the tip of the pipette from the diluting fluid, then draw the contents of the capillary portion of the tube up into the counting chamber coating the sides of same. The pipette is then filled by drawing up fresh puncture fluid and shaken five minutes, allowed to stand fifteen to twenty minutes, shaken again, and counted.

Pathologic Variations in the White Cells.—The cell-content of the cerebrospinal fluid is an extremely sensitive index of the state of the meninges.

Acute Meningitis.—Purulent fluids may contain from 4000 to 40,000 white cells per cubic millimeter.

Tubercular meningitis will average between 200 and 300 cells per cubic millimeter, although it may be as high as 20,000.

Syphilitic meningitis, tabes, paresis, usually give more than 10 and less than 100 cells per cubic millimeter.

[4] Arch. Int. Med., vol. vii.

Caution.—It must be borne in mind that the occurrence of a slight increase in the number of white cells may be the result of a previous puncture. This question should be ascertained before arriving at final conclusions in any case.

It must be remembered, further, that in cerebrospinal fluid kept at room-temperature for a short time the cells rapidly degenerate so that they are often unrecognizable after a lapse of a few hours, even if they have not entirely disappeared.

Differential Count.—In general, should the pressure of the fluids be between normal limits, and the cells less than 10 per cubic millimeter, it is unnecessary to make a differential count. Under these circumstances lymphocytes alone are present. The differential count should be accomplished speedily after removal of the fluid to prevent alteration due to decomposition. The fluid which has been allowed to fall into the centrifuge tube should be revolved rapidly for from ten to thirty minutes. This completed, the tube should be carefully removed from its metal sheath to avoid dissipating the cells. To transfer the sediment to a slide or cover-glass the fluid must be drained away by slowly and steadily inverting the tube. While the tube is still inverted the slight portion of fluid remaining in the end of the tube is taken up on a capillary pipette and blown out on to the previously cleaned glass. This is air-dried, when it is ready for fixing or staining (for methods of staining for differential count see section on "The Blood").

Lymphocytes alone (and perhaps occasionally a large, flat endothelial cell) are normal to the fluid. In the differential count from the stained specimen, it is necessary to count and classify the polymorphonuclears, the mononuclears, and endothelial cells. Mast cells, eosinophiles, tumor or nerve cells, have rarely been detected, and must still be considered exceptional, if not doubtful. The relationship of the polymorphonuclears and the lymphocytes is alone of importance in our present state of knowledge.

Polymorphonuclears are indicative of an acute process, while the mononuclears speak for chronicity. The mononuclears (lymphocytes of blood) appear to be particularly numerous in tubercular meningitis and cerebrospinal syphilis.

CHEMICAL EXAMINATION.

Determination of Protein Content.

The fluid remaining after the bacterial examination, and that supernatant after centrifugation, may be devoted to the proteid determination. An albumin and a globulin have been found in the cerebrospinal fluid; for clinical purposes their combined content usually is determined. The acetic acid boiling-test may be applied here, provided the examiner is familiar with the normal amount of proteid as represented by the white cloud formed in the upper half of the tube. For a rough quantitative determination a very narrow test-tube may be marked off in lengths to correspond to those of the Esbach tube. The Esbach reagent is used as in the similar test for albumin in urine. This test will serve to show the proteid variations from time to time in a given case.

Ross-Jones Test.—The technic of the Ross-Jones test is a refinement of the above and is of greater value, because it demonstrates an increase in the globulin content, which fails to react to this test when in normal amount. The test: 2 cubic centimeters of a saturated solution of pure ammonium sulphate (saturated by the aid of heat) are placed in a test-tube and over this is laid very carefully 1 cubic centimeter of cerebrospinal fluid. A positive reaction is shown by a white ring which forms at the zone of contact within three minutes. This ring is best seen by indirect illumination before a dark background.

Noguchi's Butyric Acid Test.—One part (0.1 or 0.2 cubic centimeter) of spinal fluid is mixed with 5 parts (0.5 cubic centimeter) of a 10 per cent. butyric acid solution (only chemically pure may be used) in physiologic salt solution. This mixture is heated to boiling and immediately 1 part (0.1 cubic centimeter) of normal (4 per cent.) sodium hydrate solution is added and the mixture again boiled for a few seconds. A positive test is shown by a definite flocculent precipitate which occurs immediately or within two hours. A faint cloudiness may be seen in normal fluids. The fluid tested must not contain blood. The test is of decided clinical value. It appears regularly in the cerebrospinal fluid of patients with syphilitic and parasyphilitic affections and also in all cases of inflammation of

the meninges caused by such organisms as the meningococcus, pneumococcus, influenza bacillus, tubercle bacillus, etc. These latter cases are, however, easily differentiated from the syphilitic affections. Normal fluid gives a turbidity, but the granular precipitate does not occur at all or only after many hours.

Albumin Estimation of Cerebrospinal Fluid.—This method, used by J. G. Greenfield,[5] is a modification of Noguchi's butyric acid reaction, in which he uses 2 cubic centimeters of fluid and the reagents in the same increased proportion. The result of the test is then poured into a graduated centrifuge tube, which gives readings to 0.1 cubic centimeter with fair accuracy; each 0.1 cubic centimeter by this method is equivalent to 0.025 per cent. Small quantities of fluid can thus be examined, and fairly reliable readings obtained from them. Greenfield found that a 0.4 cubic centimeter precipitate corresponds fairly closely to 1 part per 1000. Normal fluids give readings of 0.05 to 0.02 cubic centimeter (corresponding to 0.1 to 0.5 part per 1000). Syphilitic meningitis and parasyphilitic disease give readings up to 0.6 cubic centimeter, or 1.5 per 1000, but in no uncomplicated case did Greenfield get readings above this level.

Significance.—Although Noguchi's and the allied reactions are positive in many non-syphilitic conditions, yet they have a certain clinical value. They are not specific and, when present, do not necessarily indicate a syphilitic infection. On the other hand, they can be employed to establish or confirm a deduction based upon the clinical history and the results of the Wassermann reaction and cytodiagnosis, thus becoming of great indirect diagnostic value. Thus, a syphilitic infection is practically excluded by a negative reaction. In this respect the test has an advantage over the Wassermann reaction, in which a negative result cannot always be relied upon to indicate an absence of syphilitic infection.

Braun and Huslen[6] suggest a very simple test for distinguishing the spinal fluid obtained by lumbar puncture in meningitis from that obtained in other diseases. The reagent consists of an $\frac{n}{300}$ solution of hydrochloric acid (1 part of normal hydrochloric acid plus 200 parts of distilled water).

[5] Lancet, Sept. 7 and 11, No. 4645.
[6] Deutsch. med. Wochen., No. 25, 1912.

This is added to 1 cubic centimeter of the lumbar puncture fluid, 1 cubic centimeter of the reagent being added at a time, shaking after each addition. If no turbidity results after adding 5 cubic centimeters of the reagent, this test is negative. Over 40 lumbar puncture fluids were so tested. These investigators state that the spinal fluid in meningitis, especially if tuberculous, always gives a positive reaction, while cases of paresis give a very feeble reaction, and all others are negative.

Examination for Tubercle Bacilli.—Hemenway[7] reports a total of 135 positive results in examining 137 cases by the following technic: The fluid is collected in several test-tubes, allowing about 20 cubic centimeters per tube. The last fluid withdrawn usually shows the bacilli most readily. The tubes must not be agitated, but placed in an incubator overnight; sometimes three hours will suffice. A cobweb-like film forms, which is then removed by a platinum loop, spread flat upon the slide, and fixed and stained as usual for the tubercle bacillus. In about 4 per cent. of fluids the coagulum does not form. In such cases scraping from the slides of the test-tube mounted on slides often show the bacillus. The length of time necessary for search averages one hour, but thirty to forty minutes are generally rewarded by positive findings. For other bacteriologic and staining methods, see section on Bacteriology, chapter xi, pages 359 to 369.

ORAL SECRETIONS.

General Considerations.—The oral fluid is a mixture containing the secretions of the various buccal glands, the submaxillary, sublingual, parotid, and mucous glands. This secretion is called saliva. It is a colorless, odorless, and tasteless fluid, usually somewhat stringy and frothy and which separates on standing into two layers, the upper one of which is clear and the lower one cloudy.

Chemical Characteristics.—The normal daily amount of saliva secreted is estimated to be about 1500 cubic centimeters; this quantity, however, varies greatly. The specific gravity ranges between 1002 and 1009. The total solid content is from 2 to 12 grams. Its reaction is mildly alkaline. While the re-

[7] Amer. Jour. Dis. of Children, vol. 1, 1911.

action of the saliva is normally always alkaline, we occasionally find an acid reaction, especially in children and in the early morning hours, due to the production of lactic acid by the bacteria which are always present in the mouth. Likewise, we find an acid reaction, especially in conditions associated with acidosis.

The presence of *potassium sulphocyanate* (KCNS) is more or less characteristic of normal saliva and may be detected as follows: Collect a few cubic centimeters of saliva before a meal and allow this to filter. Add a few drops of hydrochloric acid and then a drop or two of ferric chlorid solution, when a distinct red color will develop, the depth of which will depend upon the amount of sulphocyanate present.

Ptyalin.—The most important constituent of saliva is the ptyalin. This is a definite hydrolytic ferment, converting starch into maltose through the intermediate stages of erythrodextrin and achroödextrin. This action may be readily seen by treating a little starch paste with a few cubic centimeters of filtered saliva and placing the vessel in the incubator for ten to fifteen minutes. If at the end of this time iodin solution is added, a distinct red color, instead of the characteristic blue, will appear, or else an entire absence of color change will be noted.

Saliva Test for Pancreatic Function.—A further refinement of this reaction has been suggested by Fedeli and Romaneli[8] after the demonstration of Simon that saliva, inhibited in its special activity by the gastric juice, recovered its specific properties when transferred to an alkaline medium and a little unmodified saliva or pancreatic juice added. The test is not only qualitative, but also gives a quantitative estimate of the proportion of pancreatic juice present. The test is made with 1 cubic centimeter of the individual's saliva mixed with 5 cubic centimeters of gastric juice or an equal amount of a 2.5 per cent. solution of hydrochloric acid. After thorough mixing and an interval of rest of about half an hour, add 4 cubic centimeters of a 1 per cent. solution of sodium carbonate to render the mixture slightly alkaline, and then add 20 cubic centimeters of a 10 per cent. starch paste. The whole is then kept in the incubator for two hours, at a temperature of 37° C., shaking up the mixture at intervals. It is then examined for the pro-

[8] Riforma Medica, September 13, xxv, No. 37.

portion of sugar that has been produced, and then 10 cubic centimeters of an emulsion of fresh feces (1 part stool to 3 parts distilled water) are added to the mixture, which is then replaced in the incubator, shaking it up occasionally. After twelve hours the qualitative and quantitative determination of the sugar that has been produced testifies to the intensity of the pancreatic functioning. The variation in the findings in regard to starch digestion in the first and second examinations gives an oversight of the conditions in respect to the saliva and pancreas functioning when compared with the findings in health.

Microscopic Examination.—On allowing saliva to stand it separates into two distinct layers; the upper one is clear and contains the liquid portion, while the lower is cloudy and contains the morphologic elements. A microscopic examination of a specimen of saliva that has been allowed to sediment will show many epithelial cells in the form of large, irregular, squamous plates, derived from the mucous membrane of the mouth and tongue. The characteristic cells of the saliva are the salivary corpuscles, which resemble the leukocytes, but are larger and more granular. Occasionally red blood-cells are seen, but these have no special significance, as they are derived from ulcerative or irritative conditions somewhere in the mouth or the naso-pharynx. Many micro-organisms and mold, yeast, and thread types are always present. Few of these have any direct significance, although the *Spirochæta buccalis* should be borne in mind, especially when an examination is being made of a mucous patch for the *Spirochæta pallida.* The former is differentiated from the latter by the fact that its ends lie upon a line drawn longitudinally through the center of its spirals, while such a line drawn through the pallida lies above and below its ends. (See section on parasites.) On the other hand, many pathogenic bacteria have been found in the mouth of the healthy subject, such as those of pneumonia, diphtheria, and Vincent's angina, as well as various forms of streptoccoci and the organism of thrush.

Diphtheria.—One of the most important examinations of the oral cavity consists of the detection of the diphtheria bacillus (Klebs-Löffler bacillus), as an early diagnosis of this disease frequently enables the physician to institute early anti-

toxin treatment. Such an examination should never be omitted in any case of suspected sore throat, especially when any membranous patches are present. (See also page 361.)

Vincent's Angina (Ulceromembranous Angina).—In this condition smears taken from the throat, as well as the free saliva, will be found to contain many organisms of two characteristic types, the spirilla and the long, fusiform bacilli. Usually both of these types are found together, but occasionally the spirilla are absent. The spirilla usually measure from 36 to 40

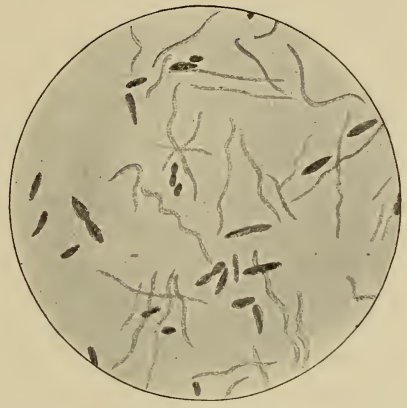


Fig. 52.—Organisms of Vincent's Angina Showing Spirillum and Fusiform Bacillus.

microns in length and ½ micron in breadth, while the bacilli are 6 to 12 microns in length and are somewhat thicker in the center than at the end. These organisms may be readily stained with Löffler's methylene-blue, gentian-violet, or dilute carbol-fuchsin; they deodorize with Gram's method. (See Fig. 52.)

Thrush.—This is a condition most commonly seen in children, but may occur in adults, especially in those with tuberculous tendencies. The saliva in this condition is usually acid and somewhat increased in amount. Microscopic examinations of the membrane show many epithelial cells, leukocytes, and much granular detritus, with a network of branching, thread-like formations, showing distinct segments. This organism is known as the *Oidium albicans*. It stains well with the ordinary aqueous methylene-blue solution.

NASAL SECRETIONS.

Normally the nasal secretion is comparatively scanty. It is clear, tenacious, odorless, salty in taste, and alkaline in reaction. It is largely composed of mucus, shows squamous and ciliated epithelium in abundance with occasionally leukocytes, large numbers of bacteria, and Charcot-Leyden and triple phosphate crystals. This secretion does not present any points for study and seems to be of pathologic significance only in infectious conditions.

THE CONJUNCTIVAL SECRETIONS.

Under normal conditions the secretion of the conjunctiva and of the lachrymal gland are of little concern. In inflammatory conditions of the conjunctiva we find certain organisms which require identification in order that proper treatment may be instituted and the proper prognosis given. The pseudodiphtheria bacillus is practically always found in made smears from the conjunctival secretion, yet it is rarely, if ever, pathogenic in this situation.

Trachoma.—A great deal of attention has lately been given to some bodies which are found in trachoma, and which are probably the long-sought cause of this infectious disease of the conjunctiva. These organisms are known as the Prowazek-Graeff bodies. They are best stained by the Giemsa stain (see Appendix), smears being made in the usual manner.

Gonococcus.—This organism may easily be found and identified in ophthalmia of this origin according to the methods outlined on page 362.

Koch-Weeks Bacillus.—This organism is the cause of true pink eye or specific conjunctivitis.

TRANSUDATES AND EXUDATES.

General Considerations.—The serous membranes are normally kept moistened by liquids whose quantity is insufficient, except in a few instances, for a complete chemical analysis to be made of them. Under pathologic conditions an abundant transudation may occur and produce accumulations of fluid in the serous cavities, in the subcutaneous tissues, or under the epidermis. Such accumulations of fluid are known as *transudates,* and

their composition is similar to the lymph, being, as a rule, poor in cellular elements and yielding little or no fibrin. These transudates should be sharply differentiated from the accumulations of fluid in the same localities, which are the direct result of inflammatory processes in the membranes lining the serous cavities and which are known as *exudates*. Exudates are generally rich in cellular elements and contain relatively more albuminous substances.

The formation of true transudates is largely a question of filtration and is controlled by the rate of blood-flow, blood-pressure, irritation of the capillary endothelium, and the variable permeability of the endothelial cells. We should expect, therefore, that the passage of dissolved substances from the blood would be regulated by the same laws that control the secretions of physiologic fluids, namely, the laws of passage of fluids through semipermeable membranes. The crystalloids would be, therefore, in approximately the same concentration as in the blood-plasma, while the colloids would be present in small quantities, the actual content being influenced by the special membrane through which the fluid passes. The condition of the blood would, hence, affect the chemical composition of such transudates, hydremic plasma yielding a fluid poorer in solids, while anhydremic blood would cause a transudate of higher specific gravity.

From a clinical standpoint a differentiation between transudates and exudates is frequently impossible, so that it is advisable to resort to aspiration of the fluid and to the chemical and microscopic examination of the material withdrawn. The important divisions of such examinations are: (1) the chemical and physical properties of the fluid; (2) the bacteriologic content, and (3) the morphologic characteristics of the cellular elements.

Obtaining the Specimen.—Whenever the fluid is to be withdrawn, either for diagnostic or therapeutic purposes, it is necessary to resort to puncture of the cavity containing the fluid. In all cases the site of puncture must be as carefully prepared as in any surgical procedure. Puncture is preferably made with a salvarsan needle with a rather large lumen, which should be carefully sterilized before use.

PHYSICAL AND CHEMICAL PROPERTIES.

Transudates are, as a rule, serous in character, usually transparent, colorless, or light yellow in color, but at times milky, reddish, or greenish, the latter practically always being observed after the fluid has stood exposed to the air. Such solutions are, as a rule, dichroic (yellow by transmitted light and green by reflected light). They are alkaline in reaction, have a specific gravity, which varies from 1006 to 1018, while serous exudates from the same cavities show a much higher specific gravity. The variations in specific gravity depend largely upon the amount of albumin present in the transudate, this practically never being over 3 per cent. and usually much lower. The chief proteins present are albumin and globulin, these being related to one another in the transudates as 1½ to 1, while in the exudates the globulin is relatively much increased. The transudates from the pleura contain the largest percentage of albumin, while edematous fluids rarely show over 1 per cent. Transudates do not coagulate spontaneously, while exudates frequently do. Glucose is present both in transudates and exudates in amounts varying between 0.04 and 0.1 per cent. The mineral constituents of transudates are somewhat higher than in the exudates, the former averaging 0.96, the latter 0.89, per cent. Pathologically, fat, blood, uric acid, and biliary pigment may find their way into both types of fluid. In diabetes an excess of sugar and the presence of acetone bodies may be detected. (Tests for these substances may be found by consulting the index.)

The *exudates* are usually straw- or lemon- yellow in color, depending on the degree of inflammation, but may range from a deep red (hemorrhagic) to a milky (purulent) shade. Biliary pigments may cause a bright green color, while various medicaments, such as methylene-blue, may produce a greenish-blue coloration. The specific gravity is almost always over 1018, the reaction alkaline, the albumin content usually above 3 per cent., reaching as high as 7 per cent., while the globulin is relatively much increased in comparison with albumin. The globulin increase is largely due to para-euglobulin. Nucleoprotein is especially abundant in purulent exudates in which the autolytic

processes are more or less marked. The total nitrogen of the various fluids varies from 0.22 to 1.38 per cent.

The following simple chemical tests have been suggested for use in differentiating an exudate from a transudate. While they have not yet been proven absolutely reliable, they are of value when taken in consideration with other clinical methods of the several tests recently advocated. To determine this often difficult point, the two following tests may be employed:—

Acetic Acid Test.—Pieper[9] says that if 100 cubic centimeters of water to which 2 drops of glacial acetic acid have been added are placed in a graduated cylinder and the fluid obtained from a puncture is instilled drop by drop, the drops will melt away in several milky streaks resembling cigarette smoke, distinctly visible against a dark background, if they are from an exudate, while if they are from a transudate they will produce no cloudiness, or in rare cases a very faint, gray opacity that can be recognized against a dark background, but will never show the milky streaks described above. If the fluid obtained by puncture is turbid it should be filtered before the test is made.

Modified Heller's Test.—S. Gangi[10] describes a test which he has found simple and reliable for this purpose. It is based on Heller's test for albumin in the urine: In a test-tube containing 2 or 3 cubic centimeters of hydrochloric acid, 3 or 4 cubic centimeters of the fluid to be examined are allowed to flow down the side. In the presence of an exudate a white cloud appears and forms a ring at the zone of contact between the two liquids. From the upper surface of this ring white clouds gather and rise, similar to the puff of smoke that rises when a lighted cigarette is struck lightly. These clouds gather to form a zone, at first at the surface, but later gradually sinking somewhat below the surface of the fluid in the test-tube, with a zone of limpid fluid above and below them down to the white ring. The ring gradually increases in width and density and finally clings to the walls of the test-tube and bubbles of gas are observed. With a transudate, on the other hand, the ring at the zone of contact is small and narrow and the fluid above persists limpid throughout or there may be no ring; after a period of turbidity the fluid

[9] Münch. med. Wochen., January 4, 1910.
[10] Riforma Medica, Naples, September, xxv, No. 38.

mixes with the acid below and forms a homogeneous, turbid, brownish fluid.

VARIETIES OF EXUDATE.

Serous Exudates.—These are clear, of a light straw color, and show a specific gravity above 1018. There is a large amount of fibrin, as shown by the dense network microscopically, which contains a few red cells, probably derived from the bleeding at the point of puncture; a few leukocytes, which may vary in type according to the kind of bacteria causing the infection, and large endothelial cells from the serous membrane lining the cavity. If the blood-cells be present in sufficient numbers to give a distinct red color to the fluid, it is termed a hemorrhagic exudate, while if a few pus-cells are found it may be called a seropurulent type.

The type of leukocytes present is usually of the polymorphonuclear variety, although other forms may be present in small numbers.

Chylous Exudates.—Such exudates show all the properties of chyle. The fluid is white and milky, contains between 1.5 and 2.5 per cent. of protein, and a considerable amount of fat, which may be demonstrated by staining with osmic acid or Sudan III or by alkalinizing with sodium hydrate and shaking out with ether.

Hemorrhagic Exudates.—This type is, in reality, a serofibrinous form containing large numbers of blood-cells. It is observed in patients with hemorrhagic diathesis, in connection with active tuberculosis, with neoplasms of the serous cavities, and following injuries to the chest or abdomen. In this form of exudate, while usually due to the tubercle bacillus, the organism is only occasionally found. The type of leukocytes (mononuclear) would be very strong presumptive evidence in favor of tuberculosis, even though no bacilli were present. If the exudate be due to a malignant growth, shreds of the tumor tissue, if found, may make a probable diagnosis.

Purulent Exudates.—These are composed either of true pus or of seropus. They are yellowish in color, thick, and occasionally tenacious, separating on standing or centrifuging into a cellular deposit and a pus-serum. The cells forming the pus

are not infrequently in a condition of advanced fatty degeneration and may contain numerous bacteria. The addition of dilute acetic acid will usually clear up the cells and render the nuclei recognizable.

Putrid Exudates.—These may be observed in various cavities of the body or in the substance of various organs, especially the liver and lungs. The material obtained by puncture is usually brownish or greenish in color, has a very offensive odor, and is usually alkaline, but may be acid. Microscopically, degenerated cells, numerous bacteria, cholesterin, fatty acid, and hematoidin crystals are observed. Bilirubin crystals and various amino-acids may be found in rupture of a hepatic abscess.

MICROSCOPIC EXAMINATION.

General Considerations.—The cytology of transudates and exudates has reference to the study of the various types of cells found in such fluids. As a rule, these investigations are most frequently carried out on the non-purulent fluid, as in the examinations of the purulent fluids we are generally concerned with the bacteria present.

Preparation of the Specimen.—The fluid when obtained should be promptly centrifuged before the formation of any clot. If it is impossible to examine it promptly, the clot should be broken up by shaking the fluid in a small flask which contains a few glass beads. Small Erlenmeyer flasks should be sterilized at 150° C. and kept ready for use. It has been found that the proportion of leukocytes obtained by prompt centrifugalization, and also after defibrinization, is practically the same.

It is necessary to centrifuge the fluid because of the small number of cells which are usually present, the concentration of the exudates rendering the examination much more rapid and satisfactory. This is especially true of serofibrinous pleuritic and peritoneal exudates, as but few elements are usually present in such exudates. If possible from 15 to 20 cubic centimeters should be withdrawn, though fair results can be obtained from 1 to 2 cubic centimeters.

The fluid should always be examined within twenty-four hours, as otherwise changes take place in the morphology of the

leukocytes which render recognition exceedingly difficult. All fluids for such examinations should be kept on ice to inhibit bacterial growth.

Centrifugalization should be prolonged (ten or more minutes), but not too rapid, for fear of destroying beyond recognition already partially degenerated elements.

Cytology of Normal Fluids.—The number of cellular elements in fluids from the various serous cavities of the body may vary from a very few to a large number. The cells observed are the red and white corpuscles of the blood, the latter of which are usually relatively more numerous than in the circulating blood and are usually largely of the polynuclear type, although mononuclear forms are frequently present. Neutrophiles and eosinophiles are present under normal conditions, the latter being relatively more abundant than in the blood. If a large number of red cells are found, an injury of the small vessels during the puncture usually explains their presence. Besides these types of cells, which are similar to those of the blood, a few endothelial cells from the lining of the cavity will usually be found.

In examining normal as well as pathologic fluids for their cellular contents 100 cells should be counted if possible and the percentage of each type thus determined. This constitutes the cytologic formula of the exudate. Such examinations may be carried out according to the technic outlined on page 62, using the Thoma-Zeiss apparatus.

Cytology of Pathologic Fluids.—The predominant cell in most infective processes is the polymorphonuclear neutrophile; the two exceptions to this are found in tuberculous and typhoid infections, when the lymphocyte is relatively much increased. This is often a valuable differential point in the study of such exudates.

THE PERITONEAL FLUID.

Characteristics of Normal Fluid.—In conditions of health there is just sufficient fluid to thoroughly lubricate the interior of the abdominal cavity. This fluid is clear, of a pale, straw color, having a specific gravity of 1005 to 1015; is slightly albuminous and, under the microscope, a drop of freshly drawn

fluid placed between a clean slide and cover-glass reveals very few, if any, formed elements.

Pathologic Exudate. — Inflammatory ascitic fluid is straw or lemon-yellow in color, and usually somewhat cloudy, depending upon the number of cells contained in it. Its specific gravity varies between 1012 and 1026, or even higher. The fluid frequently coagulates spontaneously. It shows a variable amount of albumin by boiling, and sugar, bile-pigments, urea, uric acid, and cholesterin may be demonstrated by appropriate means (see section on "Urine"). More rarely xanthin, creatinin, and allantoin may be found.

Hemorrhagic effusion occurs in cancer and tuberculosis of the abdominal cavity, and occasionally in cirrhosis of the liver.

A chylous or milky fluid is occasionally met. This is readily distinguished by examination of fresh fluid under the microscope, when the characteristic fat globules will be found.

Non-inflammatory Transudate.—In Bright's disease the ascitic fluid is usually a pale clear serum of low specific gravity, showing a minimum of cells.

In cirrhosis of the liver the color is usually darker and bile-pigment can be demonstrated.

CYST FLUIDS.

Differentiation. — Ovarian Cysts: The fluid obtained from ovarian cysts has a specific gravity usually above 1030, and is of a dark-brown, grumous appearance. Ovarian fluids are further said to contain a large number of compound granule cells which, from their frequent occurrence in this fluid, have been termed "ovarian cells." Microscopically these appear as large oval or round cells, showing dense, irregular granulations often obscuring the nucleus of the cell.

Echinococcus Cysts.—The fluid obtained by puncturing an echinococcus cyst is, in uncomplicated cases, perfectly clear, free from albumin, and contains a characteristically large amount of sodium chloride, which can easily be identified by allowing a drop of the fluid to crystallize out on a slide and examining for the square crystals of the salt. The specific gravity

varies between 1008 and 1013. Very rarely, no trace of any morphologic elements can be found. Occasionally, only a few hemosiderin or cholesterin crystals and a few fatty cells are present. Usually, however—and this is the most important point—we find, in such fluid, remnants of the scolices of the echinococcus. When these are absent, a careful search of the fluid will frequently reveal the presence of small hooklets derived from the scolices. These hooklets furnish an absolute diagnosis of the presence of the echinococcus embryos.

Pancreatic Cysts.—The puncture fluid which is obtained from pancreatic cysts varies greatly in its physical properties, depending upon the antomical nature of the cyst and the length of time the exudate has remained in the cyst cavity. The exudates which have been rapidly formed, either in traumatic cysts or in cysts connected with malignant new growths of the pancreas, are usually hemorrhagic and have a high specific gravity—that is, from 1020 to 1030.

Ferments are present in the cyst contents and may be used to identify the fluid when the exudate has existed but a short time. The contents of old cysts rarely give evidence of the presence of either the proteolytic or fat-splitting ferments.

If the fluid from a suspected cyst digests egg albumin disks or fibrin, or, as suggested by Boas, acts upon the proteids of milk, the presence of pancreatic secretion in the cyst contents may be considered probable. For the milk-test, mix the fluid with fresh milk, heat to 37° C. for several hours, precipitate the casein with acetic acid, and test the fluid by the biuret reaction. This test should always be controlled by a parallel one, using milk without cyst fluid.

PLEURAL FLUID.

General Considerations.—Physiologically there is not present enough fluid for analysis. Under pathologic conditions it may vary from one to four or more liters, and may be serous, sero-purulent, purulent, or hemorrhagic.

Non-inflammatory Transudate.—In hydrothorax the specific gravity, as a rule, is below 1015, the albumin content is small, and there is little tendency for the fluid to coagulate spontaneously. The fluid is clear or pale, straw-colored, unless

tinged with hemorrhage occurring during the puncture. The formed elements are very scarce and hard to demonstrate.

Inflammatory Exudates.—Sero-fibrinous Pleurisy: The serous exudate is abundant, and flakes of fibrin are present in the fluid, appearing as fibrillated flocculi. The actual amount of fluid varies greatly. It is of a citron color, either clear or slightly turbid from the fibrin and formed elements present. In some cases the color is dark-brown, usually due to altered blood contained in it. Specific gravity below 1020.

Microscopically, we find a variable number of leukocytes and red blood-cells, and some swollen endothelial cells and bacteria.

Purulent Pleurisy.—The specific gravity is usually above 1020. The fluid has a heavy, sweetish odor, which, if the infection be due to a penetrating wound, may be fetid. The fluid is essentially pus, contains a very large amount of albumin which may coagulate spontaneously after removal. Appropriate tests may show cholesterin, uric acid, bile-pigment, and sugar.

Method of Making Permanent Stained Preparation.—In fluids which are clear and show but few cells on microscopic examination of the fresh specimen, it becomes necessary to centrifuge 15 to 20 cubic centimeters in order to concentrate these formed elements. After centrifugalization in the ordinary urine centrifuge for from two to three minutes, the supernatant liquid is slowly and steadily poured off, and the remaining residue taken up in a capillary pipette from which it is blown out upon clean cover-slip and allowed to dry spontaneously (without heating). It then may be stained by any of the Romanowski stains (see section on "Blood") or the eosin-methylene-blue sequence.

Fluids which contain cells in macroscopic amount may be transferred to cover-glasses for staining without centrifugalization.

Microscopic Examination of Stained Preparations. — In hydrothorax the cells are few and mainly large, flat, of endothelial origin.

In pneumo- and strepto-coccic pleurisy there is a great preponderance of polymorphonuclears. In tubercular inflammations the lymphocyte is the predominating cell.

A large number of endothelial cells, occurring especially in sheets or plaques, usually means a mechanical effusion or transudate.

Many efforts at classification and diagnosis have been made on a limited number of examinations with a view to arriving at some definite rules for cytodiagnosis of pleural effusions. Further systematic study along these lines is, however, necessary.

The presence of red cells is usual in tuberculous processes, also in the presence of rupture of an abscess from an adjacent organ into the pleural cavity. Very frequently red blood-cells, in a pleural effusion, are due to the traumatism inflicted at the time of puncture. Red cells which appear in the first few centimeters drawn are most likely of this origin.

The possibility of diagnosing cancer by microscopic examination of the fluid is always present, through the finding of characteristic cell masses in the fluid.

THE PERICARDIAL FLUID.

This fluid normally is of a pale, lemon-yellow color, slightly viscid, cloudy from cell detritus; occasionally it may be clear. It contains a moderate amount of albumin and certain inorganic salts. The specific gravity ranges between 1015 and 1030.

THE SYNOVIAL FLUID.

This fluid is alkaline, thick, viscid, sticky, and of a yellowish color. Physiologically the joints contain just sufficient to completely lubricate them. Under pathologic conditions the fluid, in a large joint, may amount to many cubic centimeters.

HYDROCELE FLUID.

The fluid is usually clear, of a yellow or greenish tinge. The specific gravity varies between 1014 and 1026. The fluid sometimes coagulates spontaneously. Some leukocytes are always present, and occasionally crystals of cholesterin.

X.

HUMAN MILK.

General Considerations.—It must always be remembered, in the examination of milk, that it is no simple matter to obtain a truly representative sample. The fatty matters tend to separate, and there may be great variations between different portions of the same sample. This difficulty is particularly pronounced when human milk is examined, because of changes in the milk incident to mental and nervous disturbance.

The Sample.—The sample should consist of a thorough mixture of portions taken at different times, by means of the breast-pump. Wherever possible it is well to have the woman somewhat accustomed to the use of the pump before taking any milk for examination. In all cases the sample should be thoroughly mixed before testing, by pouring rapidly from vessel to vessel.

Physical Characteristics.—Woman's milk is bluish-white in color, of sweetish taste and characteristic odor. When freshly drawn it is alkaline or amphoteric, but never under healthy conditions is it acid. The specific gravity varies between 1026 and 1036, the average being 1031 at 60° F. On the addition of acetic acid only a slight coagulum is seen, being in the form of small, fine flocculi, never in large masses as is the case with cows' milk. Besides the myriad fat-globules, may be seen large, flat epithelial cells from the milk ducts.

Composition of Human Milk (after Holt).

	Average.	Normal variations.
Fat	4.00 per cent.	3.00 to 5.00 per cent.
Sugar	7.00 " "	6.00 to 7.00 " "
Proteids	1.50 " "	1.00 to 2.25 " "
Salts	0.20 " "	0.18 to 0.25 " "
Water	87.30 " "	89.82 to 85.50 " "
Total	100.00 " "	100.00 to 100.00 " "

The composition and food value of human milk are impaired when the mother is unhealthy. It is affected injuriously when there exists undue emotional excitement. It will contain an appreciable amount of certain medicines ingested by the mother, which may affect the infant.

Examination of the Milk.—The exact determination of the composition of human milk is only to be determined by a complete chemical analysis. There are, however, many variations from the normal which the physician may readily ascertain for himself by simple methods of examination.

The Quantity.—This may be determined roughly by using the breast-pump, although this is not reliable for many reasons. With sufficiently sensitive scales the physician may, by weighing the infant before and immediately after nursing, determine whether it is getting only one or two, or four or five ounces. The average daily quantity secreted by woman is one liter or two pints.

The Reaction.—Test by litmus paper. The reaction should be *alkaline* or *amphoteric*, but never acid when freshly drawn.

A spontaneous change occurs after milk has stood for some time in a warm place; it coagulates or sours, and becomes acid. This is due to the change of milk-sugar (lactose) into lactic acid. This occurs through the agency of the *bacterium-lactis*. The casein normally is held in solution by the alkaline phosphates, the acid changes the reaction, and hence causes the casein to be precipitated.

Specific Gravity.—This may be taken by a small hydrometer which is graduated from 1010 to 1040. The specific gravity is lowered by fat, but increased by the other solids.

Microscopically, milk is found to be composed of minute brilliant, oil globules encased in a thin envelope of casein. Immediately after delivery the milk is relatively poor in casein, but rich in fatty matter, which exists in considerable amount in the form of colostrum masses. The microscope also reveals the presence of colostrum corpuscles, blood, pus, epithelium, and granular masses. Colostrum corpuscles are abnormal after the twelfth day; blood and pus are always abnormal.

Determination of the Fat.—The simplest method is by

means of the cream gauge. This consists of a graduated test-tube with a foot, and a ground-glass neck and stopper. The tube is filled to the zero mark with freshly-drawn milk and allowed to stand at room-temperature for twenty-four hours, when the percentage of cream is read off directly from the graduated scale. The relation of cream to fat is approximately five to three. Thus 5 per cent. cream equals 3 per cent. fat, etc.

Centrifuge Method.—The use of a specially graduated centrifuge tube is more accurate. These tubes may be used in the ordinary centrifuge for urine. Only 6 cubic centimeters of milk are required for this test. If carefully conducted the test is nearly as accurate as the chemical analysis. It gives results accurate to within one-fifth of 1 per cent.

In the usual apparatus two pipettes are supplied with the centrifuge tubes, one of 5 cubic centimeters capacity, marked milk, the other holding 1 cubic centimeter up to a mark, for introducing the alcoholic solution.

To determine the fat by this method, 5 cubic centimeters of the sample is introduced into the tube by means of the pipette marked "milk," 1 cubic centimeter of the alcoholic solution (solution-A) is added, and the tube well shaken. Then, by means of any large pipette, solution-B is added little by little until the tube is filled to the zero mark. It is then placed in the centrifuge and rotated very rapidly for four or five minutes. This will bring the fat to the top in a clear yellowish layer which can be read off in direct percentage by the scale on the neck of the tube.

A few drops of water may be added, if necessary, to correct the level of the top of the fluid. If the milk should be richer than 5 per cent. it will be necessary to dilute the sample with an equal quantity of water, proceed with the test as above, and finally multiply the result by two.

Solution-A consists of:—

Amylic alcohol	37 parts by volume.
Methyl alcohol	13 parts by volume.
Hydrochloric acid	50 parts by volume.

This solution may be kept for a short while; if it turns dark it is worthless. Solution-B consists of sulphuric acid, specific gravity 1832.

Composition of Woman's Milk.

	Average. Per cent.	Common healthy variations. Per cent.
Fat	4.00	3.00 to 5.00
Sugar	7.00	6.00 to 7.00
Proteids	1.50	1.00 to 2.25
Salts	0.20	0.18 to 0.25
Water	87.30	89.82 to 85.50
	100.00	100.00 100.00

Average Normal Excretion.

	Approximately.
At the end of the first week	10 to 16 oz. (300 to 500 Gm.)
During the second week	13 to 18 oz. (400 to 550 Gm.)
During the third week	14 to 24 oz. (430 to 720 Gm.)
During the fourth week	16 to 26 oz. (500 to 800 Gm.)
From the fifth to the thirteenth week	20 to 34 oz. (600 to 1030 Gm.)
From the fourth to the sixth month	24 to 38 oz. (720 to 1150 Gm.)
From the sixth to the ninth month	30 to 40 oz. (900 to 1220 Gm.)

Determination of the Sugar.—The percentage of sugar is nearly constant, so it may be ignored in the usual clinical investigation.

Estimation of the Proteids.—Method of T. R. Boggs:[1] 25 grams of phosphotungstic acid are dissolved in 125 cubic centimeters of distilled water, and when solution is complete an equal quantity of dilute hydrochloric acid is added. The solution is stable, and keeps for months in a dark bottle. The method of procedure is as follows: The diluted milk is poured into an ordinary Esbach albuminometer tube (reading from 1 to 7 grams per liter) up to the mark U. The phosphotungstic acid solution is then added up to the mark R; the tube is corked and slowly inverted twelve times, shaking being avoided. The tube is then placed in a rack for twenty-four hours, and the percentage read off at the level of the top of the precipitate. The optimum dilution for human milk is 1 in 10. If the proteid content is low a less dilution may be employed. Controlled by Kjeldahl nitrogen determinations the mean error was 0.3 per cent., the extreme 0.7 per cent. Temperatures of from 15° to 25° C. and the presence or absence of cream make no difference

[1] Bull. Johns Hopkins Hosp., October, 1906, No. 187.

in the volume of the precipitate, which attains a minimum after standing twenty-four hours, and does not alter on further standing.

A rough estimate may be made as follows: If we regard the sugar and salts as constant, or so nearly so as not to affect the specific gravity, we may form an approximate idea of the proteids from a knowledge of the specific gravity and the percentage of fat. We may thus determine whether they are greatly in excess or very scanty. The specific gravity then will vary with the proportion of proteids, directly, and inversely with the proportion of fat, *i.e.*, high proteids, high specific gravity; low fat, low specific gravity. The application of this principle will be seen by reference to the table on page 342.

TESTS FOR FORMALDEHYDE IN MILK.

Hehner's Test.—To 15 cubic centimeters of concentrated sulphuric acid in a test-tube add 1 or 2 drops of ferric chloride test solution (U. S. P.) and mix. Then pour upon this, in such manner as not to mix the layers, the suspected milk. A violet color indicates the presence of formaldehyde. In the case of cream dilute the cream with an equal volume of water, and then apply the test as above described. The violet color is sometimes produced at once, but oftener not for five or ten minutes, and sometimes not for an hour or so, depending on the amount of formaldehyde present. By this test 1 part in 10,000 or 15,000 is readily detected.

Liebermann's Phenol Test.—In the presence of small traces of formaldehyde, distil off from the milk a few cubic centimeters and add to this 1 drop of very dilute aqueous phenol solution. Then pour this mixture slowly upon concentrated sulphuric acid in a test-tube so as to form a layer. A bright crimson color appears at the zone of contact. This may occur with as little as 1 part in 200,000 and with ease in proportions of 1 to 100,000. There is a milky zone above the red color, and, if more concentrated, there will be a whitish or pinkish precipitate. Sometimes the zone will appear, only in about one hour, and be $\frac{1}{10}$ inch below the line of contact.

XI.

BACTERIOLOGIC METHODS.

By a gradual process of evolution and development the field of clinical medicine has so enlarged its borders that to-day the laboratory worker no longer confines his investigations to examination of the blood, urine, and sputum, etc., but must possess a working knowledge of bacteriology and be familiar with bacteriologic technic to the extent that he may be able to obtain, differentiate, and recognize the commoner pathogenic organisms. This section will confine itself to a brief outline and discussion of the essentials of laboratory technic, and to a brief description of the more commonly encountered pathogenic micro-organisms. No effort has been made to make this section either exhaustive or complete, the author believing that a work of this type is more valuable if maintained within a small compass, even if the laboratory worker is occasionally driven to consult larger works, devoted entirely to bacteriology, in his search for some particular organism or technical detail.

STERILIZATION.

General Considerations. — Acquaintance with the fundamental principles of sterilization and of disinfection are absolutely necessary to the successful performance of all bacteriologic investigations. The term *sterilization,* as commonly employed, implies the absolute destruction of bacterial life by heat, while the term *disinfection* is commonly applied to accomplishing the same end through the agency of chemical substances capable of destroying bacterial life.

Strictly speaking, the term sterilization implies the complete destruction of the vitality of all micro-organisms that may be present in or on the substance or substances to be sterilized. Such a result can obviously be accomplished by either thermal or chemical means, while disinfection need not, of necessity, de-

stroy all living organisms that are present, but only those having the power of infecting or of producing disease, and may or may not, as the case may be, cause complete destruction of bacterial life, as in sterilization. It is, therefore, possible to accomplish both disinfection and sterilization by either chemical or thermal means.

In the laboratory the employment of these different terms depends upon and is governed by circumstances. It is, of course, essential that all culture media should not only be absolutely free from all bacteria, whether they are pathogenic or not, but also from their spores. In a word, they must be sterile. At the same time it is equally essential that the original chemical composition and physical properties of the media should remain unaltered by the process. It is self-evident, therefore, that sterilization of such substances by means of chemical agents is out of the question, for while this method would destroy all bacterial life, it would not only alter the chemical composition of the media, but by becoming inseparably mingled with the media, would, by its continued prsence, effectually prevent the growth of bacteria in the material for all time; that is to say, after having performed its function as sterilizer, it would by its continued presence exercise its function as an antiseptic, and render the material useless as a culture medium. Exceptions to this general rule are found in certain volatile substances such as alcohol and ether, which, after having performed their bactericidal powers, may be completely driven off by the application of heat.

Sterilization by Heat.—Sterilization by means of high temperatures may be accomplished in a variety of ways: 1. By dry sterilization, which is accompanied by subjecting the articles to adequate degrees of heat in a properly constructed oven. 2. By subjecting them to the influence of live steam at 100° C. 3. By subjecting the substances to steam under pressure. When employing steam under pressure, the temperature to which the articles are subjected will depend upon the pressure developed—the greater the pressure the higher the temperature.

Sterilization by Dry Heat.—This method has the following disadvantages which limit its applicability: 1. The temperature must be relatively high and the period of exposure long as compared to moist heat (steam). 2. The penetration of dry heat

into substances to be sterilized is much less thorough than that of steam. 3. Many substances of vegetable and animal origin are rendered valueless by the temperature required for dry sterilization.

As a preliminary to sterilization, glassware, especially when new, should be thoroughly cleansed and then cleared by soaking for one hour or more in the following solution:—

Potassium bichromate	60 parts.
Water	600 parts.
Sulphuric acid	460 parts.

Successful sterilization by dry heat cannot usually be accomplished by a temperature lower than 150° C., and this temperature must be continued for not less than an hour. In general, it may be said that dry sterilization is only suited for sterilization of such substances as glassware, dishes, flasks, test-tubes, pi-

NOTE.—In the following table Holt explains the application of this principle; it must be borne in mind that the results obtained are only approximate and cannot take the place of the actual quantitative method, except as a rough guide to variations in breast milk.

	Specific gravity.	Cream, 24-hours.	Proteid (calculated).
Average	1031	7%	1.5%.
Normal variations	1028—1029	8%—12%	Normal (rich milk).
Normal variations	1032	5%—6%	Normal (fair milk).
Abnormal variations	Low (bel. 1028)	High (ab. 10%)	Normal or slightly below.
Abnormal variations	Low (bel. 1028)	Low (bel. 5%)	Very low (very poor milk).
Abnormal variations	High (ab. 1032)	High	Very high (very rich milk).
Abnormal variations	High (ab. 1032)	Low.	Normal or (nearly so).

pettes, etc., and for such metal instruments as are not injured by heat.

Steam or moist heat sterilization possesses great penetrating power, and is much more rapid and thorough than the above method, and, further, it is far less likely to destroy the material so treated. This method should be employed for sterilizing all culture media, fabrics, cotton, wood, and organic material in general.

Aside from the relative applicability of the two methods, their mode of action toward the organisms to be destroyed is very different. The penetrating power of steam renders it far more efficacious than dry heat. The spores of several organisms which are destroyed by exposure to steam for a few minutes, resist the destructive action of dry heat at a higher temperature for a longer period of time.

The method of applying heat for sterilization depends chiefly upon the character of the substances to be sterilized. The application of dry heat is always continuous, *i.e.*, the objects to be sterilized are simply exposed to the proper temperature for the requisite time necessary to destroy all living organisms and their spores which are either in or upon them. With steam, on the other hand, the articles to be sterilized are frequently of such a nature that prolonged sterilization would injure them. For this reason it has been found desirable to subject such objects to the influence of steam intermittently for a number of short periods.

The PRINCIPLE involved in the intermittent method depends on the differing powers of resistance to heat displayed by different organisms in different stages of their development. During the life of many bacilli they enter a stage during which their resistance to both chemical and thermal agents is materially increased. This increased power of resistance is possessed by the organisms when they are in the spore or resting stage. Some spores of certain organisms have been encountered which retain the power of germination after an exposure of more than an hour to the temperature of boiling water. This difference in the thermal heat-point of bacteria and their spores is taken advantage in the process of sterilization known as the fractional or intermittent method.

As all culture media depend for their usefulness upon more or less unstable organic compounds, the effort of sterilization is to destroy the organisms in the shortest possible time by exposure to least possible amount of heat. This is accomplished by subjecting them to a temperature at a time when the bacteria are in the vegetative or growing stage. In order to develop any existing spores the media, during the intervals between sterilization, should be kept under such conditions of temperature and moisture as will favor the process of vegetation (room-temperature).

During the first application of heat the mature vegetative forms are destroyed, while certain spores which may be present resist this treatment and survive the temperature. Now the sterilization is discontinued and the media is allowed to remain for a time, usually twenty-four hours at room-temperature. During this time those spores which resisted the first heating have conditions favorable to germination. A second short exposure kills this crop of bacilli, when a second rest, followed by a third short exposure, kills the remaining organisms and the media will usually be found sterile.

It should be remembered that while all spores which are present are not killed by the first exposure, still their power of germination may be so inhibited by the exposure to 100° C. that their germination is delayed, that they cannot possibly germinate during the twenty-four hours' intervals.

Experiment has shown that the fractional process gives the best results when the objects are subjected to the action of live steam (steam at ordinary atmospheric pressure) for fifteen minutes on each of three consecutive days, and that during the intervals the cultures should be maintained at a temperature between 25° and 30° C. The substances thus treated will remain sterile for an indefinite time, provided they are not exposed to the re-entrance of micro-organisms.

An occasional exception will be noted when, after careful treatment as above outlined, certain species of spore-forming bacteria will not have been entirely destroyed by this method. These are usually of the non-pathogenic group of the so-called soil organisms.

Finally it must be born in mind that this method is only

applicable to substances capable of presenting conditions favorable to spore germination, and that dry substances, such as instruments, apparatus, or organic materials, in which decomposition has set in, where the natural conditions favorable to spore germination are absent should not be treated by this method, but must be subjected to higher temperatures for longer periods of time.

Intermittent Sterilization (at low temperature). — The process of intermittent sterilization at comparatively low temperatures is based upon the principle outlined above, but differs in the details of its application as follows: 1. It requires a greater number of exposures to accomplish complete sterilization. 2. The temperature at which it is accomplished is not above 68°-70° C.

It is employed for sterilization of easily decomposible materials and those which would be rendered useless by the application of steam at 100° C., but which are unaltered by the temperature employed. This method is applicable for sterilization of certain albuminous media where it is desirable to retain their fluid condition during sterilization and which would be coagulated by exposure to higher temperature.

This process requires that the temperature employed should be between 68°-70° C., and that an exposure of one hour should be made each of six consecutive days. During the intervals the material is kept at a temperature between 25°-30° C. to favor germination of any spores that may be present.

Direct Steam Method.—Sterilization by means of steam is also accomplished by what is known as the direct or continuous method. By this process both mature organisms and their spores are destroyed by a single exposure to steam at zero pressure—live or steaming steam. The sterilization is accomplished by a single exposure of one hour.

Steam-Pressure Method (by Autoclave). — By employing steam under pressure we are able, by increasing the temperature, to materially shorten the time necessary to accomplish complete sterilization. By employing a pressure of approximately one atmosphere (15 pounds) a temperature of about 122° C. is obtained. This is sufficient to accomplish complete sterilization by one exposure of fifteen minutes.

When this method is employed it will occasionally be found that the coagulating power of gelatin is reduced, and that it becomes slightly cloudy, while in agar-agar a fine, flaky precipitate is noticed. For accurate time and temperature exposures this is a very uncertain method. Obviously the material is subject to active temperatures during the heating up as well as during the cooling process, besides the actual time during which the maximum pressure is maintained. Also, if great care is not

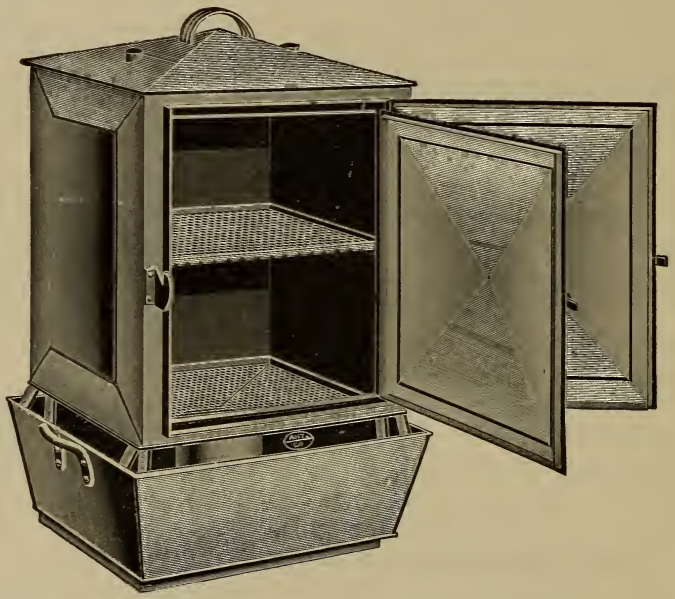


FIG. 53.—ARNOLD STEAM STERILIZER. (A. H. T. Co.)

observed to prevent premature opening of the autoclave, a sudden, rapid ebullition of the fluid occurs which, in the case of test-tubes, the media may completely boil away.

While this method of sterilization is not well suited for delicate experiments where a definite time exposure to definite temperature is of importance, still, for general laboratory purposes, it has much to recommend it in the way of a time saver, and in the certainty with which sterilization is accomplished. The apparatus designed to sterilize under pressure is termed an autoclave.

Practical Application of the Method. — 1. The Arnold Steam Sterilizer (Fig. 53) : This is probably the best sterilizer for general laboratory purposes, since it is simple and economic in its operation. The difference between this apparatus and the original sterilizer devised by Koch is that it provides for the condensation of the steam after its escape from the

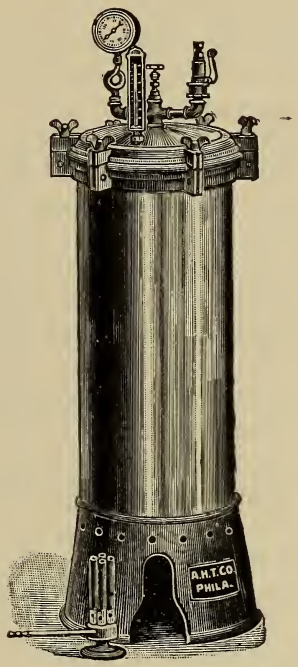


Fig. 54.—Autoclave. (A. H. T. Co.)

sterilizing chamber, and returns the water of condensation automatically to the reservoir.

2. The Autoclave.—The advantage of this method is so well recognized that its use has practically superseded the intermittent method with live steam, except when the temperature developed by the autoclave is sufficient to destroy the materials subjected to it. By this plan sterilization is accomplished in fifteen minutes by exposure to steam under the pressure of one atmosphere.

The autoclave (see Fig. 54) embodies the same principle as the steam sterilizer. It provides for the generation of steam within a chamber capable of being hermetically sealed after the introduction of the substances to be sterilized. The chamber is fitted with a safety-valve arranged for regulating the degree of pressure. A thermometer passes through the wall and enters the chamber, thus allowing the temperature to be followed and the pressure properly regulated.

Hot-Air Sterilizer.—The hot-air sterilizers employed for this work are simply double-walled boxes of Swedish iron, having a double door and a copper bottom. They are fitted with proper openings in the walls to permit circulation of the heated air. The heat is obtained from the flame of a Bunsen burner applied directly to its bottom. These bottoms are usually constructed of copper, and because they readily burn out, are now made so that they may easily be replaced. Properly constructed sterilizers with removable bottoms may now be obtained and should be sought when purchasing, as they will save much annoyance and not a little expense.

Sterilization by this method is accomplished in from a half to one hour at a temperature of from 150°-180° C.

CHEMICAL STERILIZATION AND DISINFECTION.

It is possible by means of certain chemical substances to destroy all bacteria and their spores, or by the same means to remove all their pathogenic properties; in other words, to disinfect them.

When it is desirable to use chemical disinfection in the laboratory, successful results may be obtained by employing a 33- to 40-per-cent. solution of carbolic acid. Under ordinary circumstances this will accomplish the result in from twenty minutes to half an hour. It is, however, not reliable for the destruction of spores of resistant organisms, such as the spores of the anthrax bacillus.

All materials and issues containing infectious organisms should be burned and all other cloths, test-tubes, flasks, dishes, etc., should be boiled in a 2-per-cent. soda (ordinary washing soda) for a half-hour, or should be exposed in the steam sterilizer for the same length of time.

Intestinal evacuations are best disinfected with chlorinated lime, which should contain at least 0.25 per cent. of free chlorin. This solution should be mixed in equal parts with the material to be disinfected, and then should be allowed to stand for one or two hours before being disposed of.

Sputum in which tubercle bacilli may be present, as well as vessels containing it, and the eating utensils of tuberculous patients, should be boiled with a 2-per-cent. soda solution for from a half to one hour, or should be exposed in the steam sterilizer for the same period.

PREPARATION OF CULTURE MEDIA.

Bouillon.—Five hundred grams of freshly chopped, lean beef, free from fat and tendons, are soaked in a liter of water for twenty-four hours, during which time the temperature of the mixture is kept low by surrounding it with ice.

At the expiration of this time the mixture should be strained through coarse muslin until a liter of fluid has been recovered. To this now add ten grams of dried peptone and five grams of common table salt. It is then to be rendered neutral or slightly alkaline by the addition of a few drops of a saturated sodium carbonate solution. The flask containing the mixture is then placed either in the steam sterilizer or upon a water-bath and kept at the boiling point until all the albumin has been coagulated, and the fluid portion is clear and of a pale-straw color. It is then filtered through a folded filter-paper and finally sterilized in the steam sterilizer by the fractional method (see page 345). This is the original method of Koch which has been modified and improved in the following ways:—

Neutralization.—Ordinarily this is accomplished by the addition of a saturated solution of sodium carbonate, and the reaction determined by red and blue litmus paper. This sodium carbonate solution is not so good, however, as a strong solution of sodium or potassium hydroxid, because the carbonic acid arising during the process of neutralization with the sodium carbonate frequently produces a temporary acid reaction which later disappears on boiling. Exact titration with an 0.4-per-cent. solution of sodium hydrate, obviates this difficulty. The process

is applied only after the bouillon has been deprived of all its coagulable albumin by boiling and has again been reduced to room-temperature.

TECHNIC.—First ascertain the exact volume of the fluid. From this sample take exactly 5 or 10 cubic centimeters and add a few drops of a 1-per-cent. alcoholic solution of phenolphthalein as an indicator. The 0.4-per-cent. alkaline solution is placed in a graduated burette, and the solution to be tested in a porcelain dish or casserole. Now add the alkaline solution, drop by drop, until the bouillon turns a faint rose color. A second measured quantity of bouillon is treated in the same manner as a check, and if the amount of sodium hydrate solution required to cause neutralization is the same or only slightly different, a simple calculation will indicate the amount of soda solution required to neutralize the bulk of the medium. Thus, if for 10 cubic centimeters of the bouillon would be required 1.5 cubic centimeters of the 0.4-per-cent. solution of sodium hydrate, then for the remaining 980 cubic centimeters (original volume 500 cubic centimeters), it would require 98 times 1.5 cubic centimeters of the soda solution to neutralize the total amount of bouillon, or 147 of the 0.4-per-cent. sodium hydrate solution would have to be added to the 980 cubic centimeters of bouillon to accomplish neutralization.

To avoid over-diluting the bouillon by the weak alkaline solution, it is better to employ for this purpose a 4.0-per-cent. solution of NaOH, of which only 14.7 cubic centimeters would be required.

Not infrequently the filtered neutralized and sterilized bouillon will be found to contain a fine flocculent precipitate. This may be due either to an excess of alkalinity or to incomplete precipitation of the albumin. The former may be corrected by the addition of a little dilute acetic or hydrochloric acid, follower by a second boiling, filtering, and sterilization. If due to imperfect precipitation of the albumin this may be corrected by reboiling and filtering.

2. The substitution of prepared meat extract for the fresh extract is now almost universal. Any good stock meat extract will answer the purpose, and should be used in the strength of from two to four grams to the liter of water. Peptone and

sodium chlorid are added, as in the original method of preparation.

The advantages of the meat extract are a decided shortening of the time required for preparation of the media, and the production of a more uniform material.

Nutrient Gelatin.—In making nutrient gelatin the bouillon is prepared first as outlined above, except that the reaction is corrected only after the gelatin has been completely dissolved. The reaction of the gelatin of the shops is frequently quite acid, entailing the addition of considerable more alkaline solution than required for the bouillon alone.

The gelatin is added in sufficient quantity to make a 10- or 12-per-cent. solution. Its complete solution is accomplished either on a water-bath or over the bare flame. In the latter instance it must be constantly stirred to prevent burning the gelatin on the bottom of the pan. When the gelatin is completely dissolved it can readily be filtered through an ordinary folded filter paper in a glass funnel. It not infrequently happens that in spite of the most careful technic the filtered gelatin is not perfectly clear; then clarification is required. For this purpose the mass must be redissolved, and when the temperature is between 60° and 70° C. the white of an egg, which has been beaten up with about 50 cubic centimeters of water, is added and thoroughly mixed in. This mixture is then again brought to the boiling point until coagulation of the egg albumin occurs. This process results in large, flaky coagula of albumin, which it is best not to break up, as fine flakes of albumin will clog the filter-paper and materially interfere with the process of filtration.

The filter-paper should always be moistened before filtering. If this is not done, the pores of the filter-paper will become clogged with gelatin and coagulated albumin, which will greatly interfere with rapid filtering.

Gelatin should not, as a rule, be boiled for more than fifteen minutes, nor left in the steam sterilizer for more than thirty minutes, otherwise its property of solidifying will be impaired.

As soon as the preparation of the gelatin is completed it should be sterilized in the steam sterilizer for fifteen minutes on

three consecutive days. The mouths of the containing flask or test-tubes should be completely plugged with raw cotton.

Nutrient Agar-Agar.—The preparation of this is difficult and tedious, and frequently fails from lack of patience or of experience, or both. Every worker has some slight modification of his own, but if, according to Abbott, the following directions are carefully carried out, the product will usually be satisfactory.

Prepare bouillon in the usual way. As agar-agar reacts neutral or faintly alkaline, the neutralization of the bouillon may be accomplished before the agar-agar is added. Finely chopped or powdered agar-agar is added to the bouillon in the proportion of 1 to 1.5 per cent. The mixture is then placed in an agate or earthen-ware boiler, and the height of the fluid, before boiling, marked upon its inside. If a liter of the medium is being made add about 275 cubic centimeters or more of water, and boil slowly for about two hours or until the excess of water that was added has been evaporated. Do not allow the fluid in the vessel to fall below the original level. If this occurs water must be added to bring the final amount up to one liter. At the expiration of two hours remove from the fire and cool rapidly by immersion in a pan of cold water. Stir constantly until the temperature of the mass has fallen to about 70° C., then add the white of one egg that has been previously beaten up in about 50 cubic centimeters of water. Mix this in well and allow to boil for half an hour longer, keeping the fluid up to the original one liter mark. The fluid is now easily filtered at room-temperature through a heavy folded filter. If properly prepared and filtered through a properly folded filter-paper, it should pass through at the rate of one liter in from fifteen to twenty minutes.

Glycerin Agar-Agar.—The nutrient properties of agar-agar for certain organisms is greatly increased by the addition of 5-per-cent. glycerin. If glycerin is added to the agar-agar, it should be done after filtration and before sterilization.

If after filtration the medium is found to contain flocculi, investigate the reaction. If it is quite alkaline it must be neutralized, boiled, and filtered again. If the reaction is neutral

or only faintly acid, dissolve and again clarify with egg albumin as directed. All media must be neutral or only faintly alkaline to litmus, as but few organisms develop well on acid media.

Dextrose and Lactose Litmus Agar.—One per cent. of dextrose or of lactose may be added to hot sugar-free agar broth before sterilization. Before using, sufficient of a 1 per cent. solution of sterilized litmus is added to produce a 5 to 8 per cent. solution of the same in the culture medium. This addition should be tested for sterility before use.

Blood-serum Media.—For small quantities of blood-serum for culture media purposes, blood may be obtained from small animals under such aseptic precautions as will guard against gross contamination. For laboratory purposes, where large amounts are required, it is best obtained from the slaughter houses. Under these conditions a certain amount of contamination is unavoidable, though its extent may be limited by observing certain precautions.

Koch's original method, with slight variations, follows:—

The animal from which the blood is to be obtained should be suspended by the hind legs so that its head is a few feet from the floor. The head should be held back when, with one sweep of a sharp knife, the throat is completely cut through. The blood as it spurts from the vessels should be collected in large glass jars which have been previously sterilized and dried with alcohol and ether. The jars should be provided with close-fitting cover and clamps capable of hermetically sealing them (large museum specimen jars will be found very satisfactory for this purpose). From two-gallon jars of blood there is usually recovered from 500 to 700 cubic centimeters of clear serum.

The jars having been filled with blood, their covers are replaced loosely and then they are allowed to stand quietly for about twenty minutes until clotting has begun. At the expiration of this time a clean glass rod is passed about the edges of the surface of the forming clot to break up any adhesions that have formed to the sides of the jar. The covers are now replaced and clamped down tightly, then with as little agitation as possible the jars are transferred to an ice chest, 40° F., where they should remain for from twenty-four to forty-eight hours. When the jars are removed from the ice chest a firm clot

will be found in the bottom of the jars. The serum is drawn off with a sterile pipette or syphon, and transferred to sterile glass cylinders. These cylinders are now placed on ice for another twenty-four hours, when the corpuscles will have settled to the bottom, leaving the serum above quite clear. This is then ready to be pipetted off into sterile test-tubes, about 8 cubic centimeters in each tube, or into flasks of 100 cubic centimeters' capacity. It is now ready for sterilization. This is accomplished by the intermittent method at a temperature of 70° C. for a period of one hour on five consecutive days. During the intervals it should be kept at room-temperature. After sterilization the tubes may be allowed to remain fluid or they may be coagulated by a short exposure to 80° C. For solidifying, the tubes should be placed in an inclined position in order to secure the greatest possible surface from the quantity of serum employed.

The process of solidification requires constant attention if good results are to be obtained. No rule can be laid down for the time required to accomplish it, as this is not constant. Too high temperature and too rapid solidification results in an opaque and inelastic medium.

When solidification is complete the tubes may be retained in a vertical position, and unless for immediate use must be protected from drying. This may be done by burning off the superfluous ends of cotton and covering them with sterile rubber caps; or what is just as satisfactory and far cheaper, sterilized corks may be pushed down upon the cotton plugs.

Owing to the employment of large quantities of serum, principally for the detection of diphtheria, the tedious method of Koch has been largely superseded by a number of more rapid modifications.

Method of Councilman and Mallory.—By this method the serum is more quickly prepared. Rigid precautions against contamination of the blood during collection are not necessary, and the resulting medium, while neither transparent or translucent, fully meets the ordinary requirements of bacteriology.

By this method the serum is decanted directly into sterile test-tubes as soon as obtained; it is then firmly coagulated in the slant position by exposure in a dry-air sterilizer at from 80° to 90° C. It is then immediately sterilized in the steam sterilizer

at 100° C. for fifteen minutes on three consecutive days, as is the case with other media.

Loeffler's Blood-Serum Mixture.—This mixture consists of one part neutral meat-infusion bouillon, containing 1 per cent. of grape-sugar and three parts blood-serum. The mixture is placed in test-tubes, sterilized, and solidified in exactly the same manner as described under blood-serum preparation, except that it requires a longer time and a higher temperature for coagulation.

PREPARATION OF TUBES, FLASKS, ETC., FOR CULTURE MEDIA.

While the media are in the course of preparation it is well to get the tubes, flasks, pipettes, etc., ready for their reception. These must be absolutely sterile. To this end both old and new tubes should first be boiled for a half-hour in a 2-per-cent. soda solution, then carefully swabbed out with appropriate bristle brushes. After rinsing in clear water they are immersed in a 1-per-cent. solution of hydrochloric acid for a few minutes, then rinsed again and stood, round end up, to drain. When dry they are plugged with raw cotton carefully inserted so that there are no cracks or openings in the occluding cap. The plug should fit neither too loosely nor too tightly, but should fit firm enough to hold the weight of the tube when lifted by the protruding cotton.

The tubes thus plugged are then placed upright in wire baskets and heated for one hour in the hot-air sterilizer to a temperature of 150° C. Tubes so prepared, if undisturbed, will remain sterile for an indefinite period.

Filling Tubes.—The tubes are best filled with the aid of a separating funnel, though if not convenient the tubes can, with a little care, be successfully filled directly from the flasks. It is not necessary to sterilize the funnel as the media in the tubes is to be sterilized as soon as they are filled. In any case, care should be observed to prevent any of the medium from coming in contact with the mouth of the tubes, which would cause the cotton plugs to adhere to it, making them hard to remove, presenting a very untidy appearance and materially interfering with subsequent manipulations.

After filling, the tubes are ready for final sterilization. This

is accomplished in the steam sterilizer by the three-day fractional method.

TECHNIC FOR PLATES AND PETRI DISHES.

Plates.—The plate method can be employed with both agar and gelatin, but cannot be practiced with blood-serum, because the latter when once it is solidified cannot again be rendered liquid.

Plates are usually referred to as a set. This term includes three separate plates each representing a mixture of the organism in a state of greater dilution. The plates are numbered 1, 2, and 3. A set of plates may be prepared as follows: Three tubes, each containing the requisite amount of gelatin or agar-agar, are placed in a water-bath and warmed until the medium is fluid. Agar-agar becomes fluid at about the temperature of boiling water; gelatin is fluid between 35° and 40° C. In the case of the agar-agar tubes, after liquefying they must be cooled to 40° C., at which temperature they remain fluid while the organisms are introduced. If this cooling is omitted the temperature of the medium when the organisms are inoculated will be sufficiently high to destroy their vitality.

The medium now being liquid and of a proper temperature, the material containing the organisms is taken up on a sterile platinum wire-loop and transferred to tube 1, where it is thoroughly disintegrated and mixed by rubbing against the side of the tube. The more carefully this is done the more uniform will be the distribution of the organisms and the better the final results. The loop is now again sterilized by passing through the flame, and when cool three loops full from tube 1 are transferred to tube 2, where they are carefully stirred in. Again, the wire is sterilized and the same manipulation carried out between tubes 2 and 3. This completes the dilution.

During these manipulations, which must be done rapidly if agar is employed, the temperature of the water bath must be kept between 39° and 43° C. If the temperature falls below 38° C. the agar-agar will become solidified and can then only be reliquefied by the application of heat sufficient to destroy the organisms introduced.

After inoculation the contents of these tubes are poured out

upon the sterilized plates, cooled, and incubated for twenty-four or forty-eight hours.

Petri Dish Method.—This process materially simplifies the original technic of the plate method. It consists in substituting for the flat plates of glass small, round double-glass dishes having about the same surface area as the original plates. The inoculated and liquid media is poured directly into these, their covers are immediately replaced, and they are set aside to cool. In all other respects the process is the same as Koch's original plate method. These dishes have vertical sides which prevents overflowing of the medium. A convenient size for this method measures about 12 centimeters in diameter, but dishes of other sizes are easily obtainable. The dishes are readily sterilizable by hot air or steam, and have the great advantages that the danger of contamination is reduced to a minimum, since after sterilizing the plates do not have to be separated until the pouring on of the medium, and then only for a moment.

THE INCUBATOR.

After the plates have been made and solidified they should be transferred to an incubator where a uniform and favorable temperature may be maintained. Various types of incubators have been devised, but since the principle and purpose of all is the same, a general description of their construction and method of employment is all that is required.

The incubator or thermostat (Fig. 55) consists essentially of a copper chamber of convenient size provided with double walls, between which heated water circulates. The incubator chamber has a close-fitting door of heat-proof construction, and usually within this is a second door provided with a glass front, which permits inspection of the interior of the incubator without actually opening the chamber, and so reducing the temperature. The whole apparatus is set upon an enclosed base and is covered with asbestos board to prevent loss of heat from radiation. In the top of the chamber is a small opening fitted with a perforated cork through which a thermometer projects into the interior. Two other openings are provided, one for the thermometer which records the temperature of the circulating water, the other for the thermo-regulator. At one side of the

apparatus is a vertical water gauge provided with an upper opening for the introduction of water, and a stop-cock below for drawing off the water when occasion required.

When in operation the apparatus should be kept full of water; otherwise, the object of the water jacket will be defeated and the temperature of the interior of the chamber will not be maintained. Heat is supplied to the incubator by a gas burner

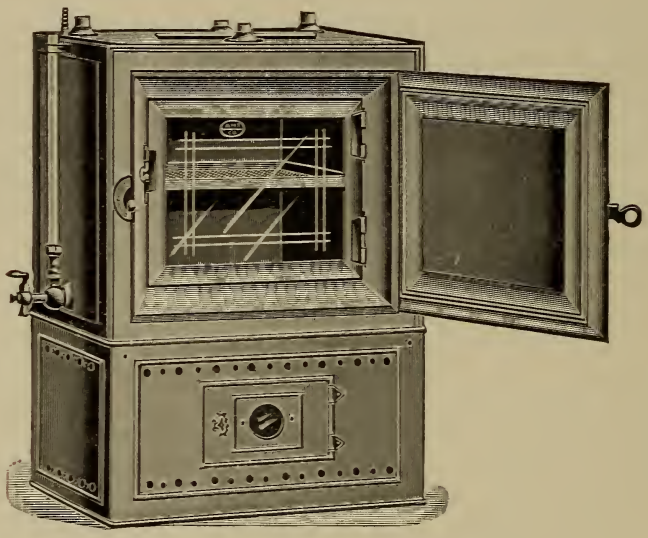


FIG. 55.—THERMOSTAT OR INCUBATOR. (A. H. T. Co.)

placed within the inclosed space below the chamber. The particular form of burner usually employed is known as "Koch's safety burner," which is so constructed that should by accident the light be extinguished, the flow of gas would be almost immediately shut off. An ordinary Bunsen burner, well protected from sudden gusts of air, will serve the purpose equally as well.

The Thermo-Regulator.—The efficiency of the thermostat depends upon the proper and uniform temperature which is maintained by the thermo-regulator. A satisfactory regulator should permit of a fluctuation of not more than 0.2° C. in the temperature within the chamber of the apparatus.

The commonest form of regulator is constructed upon the principle involving the expansion and contraction of fluids under the influence of heat and cold. By means of such expansion and contraction the amount of gas passing from the source of supply to the burner is modified as the temperature of the water in the incubator rises or falls.

For the successful employment of clinical laboratory methods, the thermo-regulator is useful, but not essential. In a room of fairly uniform temperature the flow of gas can be regulated by hand until the internal temperature of the incubator is 37° C. After this, if the water level is maintained and the apparatus protected from protracted change in the surrounding temperature, the variation in the internal temperature will be so slight that it need not be considered.

DESCRIPTION OF COMMON DISEASE-PRODUCING ORGANISMS.

The Tubercle Bacillus.—This organism has been fully discussed in chapter ii, on Sputum, as it is in this material that search is usually made for purposes of diagnosis (see pages 30 to 33). The tubercle bacillus may be found in any secretion or excretion as well as in any organ of the body. In different localities the details of its isolation and detection must naturally differ, although after the preliminary technic of isolation and specimen preparation the methods of fixation, staining, and microscopic search are similar to those already described elsewhere, and will not be repeated here.

Tuberculosis of tissues other than the lungs is probably best demonstrated by inoculation experiments carried on in guinea-pigs, and even in sputum examination, supplementary animal inoculation is desirable, when available, as a means of control. This seems almost essential to-day when early diagnosis is insisted upon, because investigators have shown that staining methods fail to give positive findings in doubtful cases, while properly conducted inoculations almost never fail.

One of the unsettled controversies which has been waged among bacteriologists and pathologists during the past six years is that of the diagnostic value of finding acid-fast bacilli in the blood. In spite of the time that has elapsed since the statement

of Rosenberger, the question of the prevalence of tubercle bacilli in the circulating blood of all cases of tuberculosis is still unsettled, the preponderance of evidence pointing to the belief that tuberculosis is not regularly, although it occasionally may become, a bacteremia.

Through the courtesy of Dr. Randle C. Rosenberger,[1] the originator of the test, I am enabled to give in full the methods employed by him in isolating these acid-fast organisms from the blood and feces.

The Method.—The Blood: Take 5 or 10 cubic centimeters of blood from a vein in the arm of the patient (after having cleansed the part thoroughly). The blood is best drawn through a large hypodermic needle fitted with a short piece of rubber tubing, which is held so as to extend into a sterile test-tube or centrifuge-tube. The tube should contain 5 cubic centimeters of a sterile 2 per cent. solution of neutral sodium citrate to prevent the least coagulation. Antiformin is now added (pure) until the blood is entirely destroyed. Only a very small quantity of antiformin is needed and should be added, a few drops at a time. The specimen is now centrifugated for twenty minutes, the supernatant fluid carefully poured off and the small precipitate carefully washed with sterile distilled water to remove the antiformin; again centrifugate, collect the precipitate on a perfectly clean slide, dry, fix, and stain for five or ten minutes with cold carbol-fuchsin; wash in water and then apply Pappenheim's solution. The slide is left in Pappenheim's solution for two or three minutes, washed with water and the stain again applied for five minutes; this procedure is repeated until the slide actually receives twenty minutes' intermittent immersion in the Pappenheim solution. Wash in water, dry and mount, or examine immediately without mounting for red bacilli.

The Feces.—Make a spread from any portion of the stool upon a clean slide; dry, fix and stain as above (for blood). If acid-fast organisms are not found by this method, take about 5 grams of the specimen and add pure antiformin, 1 part antiformin to 4 or 5 parts feces, and allow this to act for from fifteen to twenty minutes; then add sterile distilled water;

[1] Personal communication.

centrifugate; wash the sediment with distilled water; again centrifugate; collect the sediment upon a clean slide, and spread and stain as before. As a rule, by this procedure all other bacteria are destroyed, so that the only organisms found will be those of tuberculosis.

BACILLUS OF DIPHTHERIA.

From the grayish-white deposit on the fauces of a diphtheritic patient, prepare a series of cultures in the following way:—

Have prepared a few tubes of Löffler's blood-serum. Pass a stout platinum needle which has previously been sterilized into the membrane and rub it around there; then being careful that it touches nothing else, rub it carefully over the surface of two tubes. The tubes are then immediately replugged and placed in the incubator. If the case be one of true diphtheria, the tubes will be ready for examination the following day.

The blood-serum mixture is to be preferred to the ordinary plate method because the organism of diphtheria grows better on this medium than upon any other; it is also a differential method in a general sense, because other organisms do not grow well on Löffler's serum; hence a luxuriant growth at the expiration of twenty-four hours should always be considered diphtheritic until proven otherwise.

Appearance.—After twenty-four hours the tubes present a characteristic appearance. Their surfaces are marked by more or less irregular patches of white or cream-colored growths, which are usually more dense at the center than at the periphery.

Staining.—From this culture smears are made upon clean cover-slips or slides, dried and fixed in the usual way, and then stained with Löffler's alkaline methylene-blue. There will now be seen, in a typical case, upon microscopic examination, slightly curved bacilli of irregular size and outline. In some cases they will be more or less clubbed at one or both ends; sometimes they are spindle shaped or may present curved edges. They are rarely or never regular in outline. Many of these irregular rods are seen to be marked at circumscribed points in which their protoplasm is deeply stained. This irregularity in outline and

appearance is the morphologic characteristic of the bacillus of diphtheria.

THE GONOCOCCUS OF NEISSER.

On **microscopic examination,** the pus from an acute case of gonorrhea will show numerous small bodies, usually arranged in pairs. These cells will be found both within and without the protoplasm of the pus-cells. The cells containing the gonococcus are usually crowded with the organisms, though the majority of pus-cells do not contain them. This organism, on account of its frequent arrangement in pairs, is often called the diplococcus of gonorrhea. It is always found in gonorrheal pus and often persists into the convalescent stage after the external discharge has disappeared. It is easily detected in the pus of acute invasion, while in the subacute and chronic conditions its detection is often a matter of considerable difficulty.

Cultivation.—It does not grow upon the ordinary culture media, and can only be isolated in culture through the employment of special methods. Blood or blood-serum is a necessary constituent of all media for the artificial cultivation of this organism. Some investigators have been successful in growing it upon other body fluids, such as ascitic fluid, pleural effusion, and the fluid from ovarian cysts. A useful medium may be prepared by mixing equal parts of human blood-serum with ordinary sterilized nutrient agar-agar. This is accomplished by liquefying the agar, maintaining it at a temperature of 50° C. until after the mixture is made, after which it is allowed to solidify.

Distinguishing Characteristics.—1. It is seen practically always in the form of a diplococcus, having the characteristic biscuit form with the long diameters of the individual cells apposed.

2. In gonorrheal pus some organisms are practically always found within the protoplasm of some of the cells.

3. It stains readily with the ordinary staining reagents, but it is promptly decolorized by Gram's.

4. It fails to develop on the ordinary artificial media (separating it from the *Diplococcus intracellularis meningitidis,* which grows freely).

5. It has no pathogenic properties for the lower animals.

Staining.—The ordinary stains are satisfactory, one of the simplest being a 1 or 2 per cent. aqueous solution of methylene-blue, which gives a very clear picture.

Most important as a differential method is its failure to retain its color when treated by Gram's method.

MENINGOCOCCUS (MICROCOCCUS MENINGITIDIS).

This organism was extensively studied first by Weichselbaum in 1887, who described its relation to acute inflammatory involvement of the meninges. It is now recognized as the specific cause of epidemic cerebrospinal meningitis.

Morphology.—In cover-slip preparations the meningococcus appears as a diplococcus or in tetrad form. Characteristically it appears within the polymorphonuclear, these white cells often being so densely packed with the organism as to obscure the nucleus. They are readily decolorized by Gram's. This characteristic serves to distinguish them from the ordinary streptococci and from the *Diplococcus pneumoniæ*. They do not possess a capsule, but involution forms are common (Jordan).

Culture Characteristics.—They grow with difficulty on the usual culture media and are best cultivated on Löffler's blood-serum. They are of poor vitality and, unless immediately planted, efforts at culture may be unsuccessful because of the death of the organisms.

Staining Methods.—The cover-slip after proper fixation may be stained with alkaline methylene-blue or any other ordinary stain, and for differentiation the Gram method should be employed.

TYPHOID BACILLUS.

This organism has been known since 1880 and is now recognized as the specific cause of typhoid fever in at least the great majority of cases, so designated. It must be remembered that cases clinically diagnosed as typhoid fever, especially if they vary in some details from the classical picture of this disease, may be due to one of the paratyphoid organisms. This will account for the failure to obtain a specific agglutination,

and other laboratory examinations for the demonstration of this disease.

Morphology and Staining.—The typhoid bacilli are short, rather thick rods, having rounded ends, often growing in long threads. They differ from the other members of the colon group in that they are more slender and are usually longer. These organisms take the ordinary aniline stains, but less intensely than the majority of organisms. Bipolar staining is sometimes seen, and like the colon and paratyphoid group they fail to retain the color when treated with Gram's. The organisms usually possess numerous flagella, which may spring from the sides as well as from the ends of the rods. Short rods may be encountered, which possess but a single terminal flagellum. The organism is, therefore, motile, aërobic, and a facultative anaërobe. The organism does not form spores.

The motility of the organism facilitates the demonstration of the Widal agglutination reaction discussed in the chapter on Serodiagnosis (see page 370).

The organisms are usually present in the blood in patients ill with typhoid fever. This fact is of value in early diagnosis, especially when the Widal is negative, although it must be remembered that McFarland has examined the blood of typhoid patients daily throughout the course of the disease and found 10 per cent. of cases always negative.

Blood Cultures.—The following method may be employed for obtaining blood cultures in typhoid fever: A 10 cubic centimeter Leur syringe is sterilized and protected in a large glass tube. The patient's arm is constricted above the elbow by means of a rubber bag of a sphygmomanometer, until the veins at the bend of the elbow stand out prominently. The space at this point is thoroughly scrubbed with alcohol and painted with tincture of iodin. From 8 to 10 cubic centimeters of blood are then drawn up into the syringe; 1 to 1.5 cubic centimeters are mixed well with about 100 cubic centimeters of sterile broth in a flask; 1 to 1.5 cubic centimeters with about 8 cubic centimeters of sterile bile, and about 1 cubic centimeter is added to each of 3 agar plates. All of these are then incubated for twenty-four hours. If, at the end of that time, hanging drops show motile bacilli resembling typhoid organisms in their morphology

and manner of motion, subcultures are made on litmus milk, glucose agar, agar slant, broth, and peptone. It is advisable to subculture at the end of twenty-four hours, whether the hanging drops show motile bacilli or not, in which case the original cultures should be incubated for another twenty-four hours before subculturing. In most cases the earlier subculturing will hasten the diagnosis. A growth of typhoid organisms on the original blood-agar plates is the most difficult to obtain, but even then it may be the means of ruling out streptococcus and pneumococcus infections. If the subcultures show a characteristic growth in the agar slant, with no acidification of the litmus milk, no gas in the glucose agar, no indol in the peptone culture, and a characteristic motile bacillus in the broth, the diagnosis may be considered sufficiently established (Jordan).

Elimination of the Typhoid Bacillus From the Body.—The typhoid bacillus frequently gets into the secretions and is at some time during the disease present in the urine in about 20 per cent. of cases. It is occasionally found in the sputum, is almost always present in the gall-bladder, and is always found in the feces. The bacillus usually disappears from the body at about the end of the fifth week, but may occasionally remain for months in the urine and throughout life in the gall-bladder. Examinations of typhoid fever cases show that from 1 to 5 per cent. continue to pass typhoid bacilli for many months or even years. This knowledge has given rise to the recognition of a special class of individuals known as typhoid carriers. The majority of such persons are women.

CLASSIFICATION OF BACTERIA ACCORDING TO STAINING PROPERTIES.

Bacteria may be divided into two groups, according to whether they are positive or negative to Gram. By positive we mean that the bacteria retain the primary color or the gentian violet after decolorization with iodin. The term "negative to Gram" indicates that the bacteria lose the primary color after treatment with alcohol and take up the counterstain, such as Bismarck brown or carmin.

Gram's Method.—Objects are first treated with an anilin-water solution of gentian-violet which is made after the formula

of Koch-Ehrlich (see Appendix for preparation of stain). After staining in this solution for fifteen to thirty minutes the excess of stain is drained off and the film treated for five minutes with Gram's iodin solution (see Appendix). They are next transferred to alcohol and thoroughly rinsed. If careful examination at this point reveals any violet color in the film, it must again be treated with the iodin solution until all violet color is removed. After a final washing in alcohol the specimen may be mounted and examined or a counter-stain of carmin may first be employed.

Wright's Modification.—Stain for one minute in carbol-gentian-violet (see Appendix). Wash in water from thirty to sixty seconds. Lugol's solution is then allowed to act upon the specimen for one to three minutes. Wash and dry. Differentiate with anilin-xylol (2:1) to which 1.5 per cent. of acetone has been added, for one or two minutes. Wash with xylol, dry, and counter-stain with dilute carbol-fuchsin (1:10) for about one minute, during which the specimen should be warmed slightly. The specimen is finally washed, dried, and mounted for examination.

The process of decolorizing is only a relative one, some bacteria decolorizing more readily than others, so that much depends upon the intensity of the decolorizing reagent, and also upon the time during which it is allowed to act. The counter-stain method with dilute carbol-fuchsin is a differentiated process indicating those organisms that have been decolorized. All decolorized organisms by this method take on a red color. This counter-stain is of particular value where pictures of a number of bacteria are made, as in sputum examination.

The following organisms retain the violet stain by Gram's methods:—

Streptococci.
Staphylococci.
Bacillus tuberculosis.
Bacillus anthracis.
Bacillus aërogenes capsulatus.
Bacillus diphtheriæ.
Diplococcus pneumoniæ (Fränkel's).
Bacillus tetanus.

Smegma bacillus.
Lepra bacillus.
Timothy bacillus.
Saprophytic cocci of the urethra.

The following organisms are decolorized by Gram's method:—

Diplococcus meningitidis intracellularis.
Bacillus pyocyaneus.
Micrococcus gonorrhœæ (Neisser's).
Bacillus malignant edema.
Bacillus influenzæ.
Bacillus typhosus.
Bacillus choleræ.
Bacillus pneumoniæ (Friedländer's).
Morax-Axenfeld.
Colon bacillus.
Bacillus pestis.
Micrococcus catarrhalis.
Koch-Weeks bacillus.

The bacillus of Friedländer, the diphtheria bacillus, and the *Diplococcus intracellularis* are somewhat variable in their behavior toward Gram's stain, and may or may not decolorize, but the most usual type is taken in the column of the classification table.

LÖFFLER'S METHOD OF STAINING FLAGELLA.

It is essential that the bacteria be evenly and not too numerously distributed over the cover-slip.

Preparation of Cover-slip.—The glasses must be perfectly clean. Lay six cover-slips on an even surface, and place in the center of each a small drop of distilled water. From the material to be examined, transfer a minute quantity to the drop of water on the first cover-slip; from this one transfer a minute quantity to the second, and so on to the sixth. This insures a varying dilution of the organisms in the different preparation. They are then all spread, dried, and fixed in the usual way. The cover-slip preparations are next warmed in Löffler's mordant (see Appendix).

A few drops of this solution is placed upon the preparation, which is held over a low Bunsen flame until it begins to steam. It should not be boiled. After steaming for a few moments the mordant is washed off with water and then with alcohol. The bacteria are now stained in the Koch-Ehrlich anilin-water-fuchsin solution (see Appendix).

When treated in this way various bacteria behave differently, the flagella of some staining readily; others require the addition of an alkali in varying proportion to obtain the best results; others again stain best after the addition of an acid.

To meet these conditions an exact 1 per cent. solution of caustic soda in water must be prepared, and also a solution of sulphuric acid of such strength that 1 cubic centimeter will exactly neutralize 1 cubic centimeter of the alkaline solution.

For the different bacteria which have been studied by this method Löffler recommends that one or the other of these solutions be added to the mordant before using, in the following proportions:—

Of the Acid Solution.

For Spirillum concentricum, no addition of either acid or alkali.
For Spirillum cholera Asiaticæ, ½ to 1 drop to 16 cubic centimeters of the mordant.
For Spirillum Metchnicovi, 4 drops of acid to 16 cubic centimeters of the mordant.
For Spirillum rubrum, 6 drops of acid to 16 cubic centimeters of the mordant.
For Bacillus pyocyaneus, 5 drops of acid to 16 cubic centimeters of the mordant.

Of the Alkaline Solution.

For Bacillus mesentericus vulgaris, 4 drops of alkali to 16 cubic centimeters of mordant.
For Bacilllus Micrococcus agilis, 20 drops of alkali to 16 cubic centimeters of mordant.
For Bacillus typhosus, 22 drops of alkali to 16 cubic centimeters of mordant.
For Bacillus subtilis, 28 to 30 drops of alkali to 16 cubic centimeters of mordant.
For Bacillus malignant edemæ, 36 to 37 drops of alkali to 16 cubic centimeters of mordant.
For Bacillus symptomatic anthrax, 35 drops of alkali to 16 cubic centimeters of mordant.
The drops used run 22 to the cubic millimeter.

CAPSULE STAINS.

Welch's Method[2]:—

1. Cover the film with glacial acetic acid.

2. Draw off acetic acid and treat the film several times with anilin-gentian-violet.

3. Wash in 0.85 per cent. NaCl solution and examine in the same solution. Avoid the use of water at any stage. The capsule appears as a pale violet halo around the deeply stained bacterium.

Muir's Method:—

1. Prepare, dry, and fix film in the ordinary manner.

2. Flood the film with carbol-fuchsin; warm until steam begins to rise; allow the stain to act for thirty seconds.

3. Wash quickly with methyl alcohol.

4. Wash thoroughly with water.

5. Then apply the following mordant for five seconds:—

Corrosive sublimate, saturated aqueous solution	2 c.c.
Tannic acid, 20 per cent. aqueous solution	2 c.c.
Potash alum, saturated aqueous solution	5 c.c.

6. Wash off quickly and thoroughly with water.

7. Treat with methyl alcohol for about sixty seconds (the preparation should now be pale red).

8. Wash thoroughly in water.

9. Counterstain with 1 per cent. aqueous methylene-blue solution for thirty seconds.

10. Wash in water.

11. Dehydrate in alcohol.

12. Clear in xylol and mount in xylol balsam.

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[2] Bull. Johns Hopkins Hosp., 1892, iii, p. 128.

XII.

SERODIAGNOSIS.

AGGLUTINATION REACTIONS.

SPECIAL AND SPECIFIC REACTIONS.

Agglutination.—We know that in the natural infections of man peculiar changes occur in the blood-serum due to the influence of specific bacteria or their soluble toxins. Among these, the most familiar occurs in typhoid fever and is shown by the agglutination of these motile organisms in the Widal reaction.

Definition.—Agglutination in a bacterial sense refers to the clumping or precipitation of micro-organisms by the action of serum. While first employed as the Gruber-Widal reaction in the diagnosis of typhoid fever, subsequent investigations have demonstrated the applicability of this reaction in the blood of a number of specific infections.

Normal serum may agglutinate many bacteria, as typhoid, colon, pyocyaneus, and dysentery, but not the streptococcus and many others. This agglutination only occurs in low dilutions, though the typhoid has been found to agglutinate in dilutions of 1 to 30. This point is to be remembered in practical diagnosis, and care must be taken in the performance of these tests to carry them out with sufficient dilution to avoid a spurious reaction, due to a too concentrated serum.

THE SPECIFIC TYPHOID OR WIDAL REACTION.

There are two methods: 1. The macroscopic or naked-eye observation of the clumping and sedimentation of a homogeneous suspension of bacteria in a test-tube. 2. The microscopic observation of the clumping of the organisms when mixed in diluted serum and mounted in a hanging-drop preparation.

For the hanging drop it is necessary to have slides with concave depressions in the middle. A drop of the serum under

examination is placed in the center of a cover-glass, which is then placed drop-side down over the depression, its edges being sealed by vaselin or paraffin. In this preparation with the aid of a microscope the loss of motility incident to agglutination is readily observed.

Different typhoid cultures vary in their susceptibility to clumping, so that each observer should make himself familiar with the peculiarities of his own cultures.

In order to have fresh typhoid cultures always at hand it is best to transfer glycerin-agar cultures every eight to fourteen days, or otherwise cultures degenerate and die off. A bouillon culture is prepared from the water of condensation of the agar culture twenty-four hours before the test is to be made and is incubated at room temperature.

Microscopic Reaction.[1]—Five hanging-drop preparations should be made as follows:—

(*a*) One loopful of bouillon culture and 1 loopful of pooled serum. This is the control (pooled serum is serum prepared from the blood of several individuals known to be free from typhoid fever).

(*b*) One loopful of culture + 1 loopful undiluted specific serum = 50 per cent.

(*c*) One loopful of culture + 1 loopful 10 per cent. serum (1 part serum diluted with 9 parts normal saline) = 5 per cent.

(*d*) One loopful culture + 1 loopful 1 per cent. serum (1 part serum diluted with 99 parts normal saline) = 0.5 per cent.

(*e*) One loopful culture and 1 loopful 0.1 per cent. serum (1 part 1 per cent. serum diluted with 9 parts normal saline) = 0.05 per cent.

These are examined from time to time, a record being made of the time that the dilutions were made. The control should not agglutinate. The 5 per cent. dilution should remain non-agglutinated for thirty minutes before reporting a negative reaction. The 0.5 and 0.05 per cent. preparations may or may not agglutinate, depending on the potency of the serum.[2]

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[1] Eyre: "Bacteriologic Technic," 2d Edition, 1913.

[2] The Thoma-Zeiss Hemocytometer tubes may be used for diluting the serum.

Microscopic Reaction.—1. Place from 90 to 100 cubic millimeters of diluted serum into each of 3 sterile test-tubes: $a = 10$; $b = 1$ per cent., and $c = 0.1$ per cent. dilutions. In a fourth tube place the same amount of 50 per cent. pool serum.

2. Add to each tube the same amount (90 or 100 cubic millimeters) of twenty-four hours' bouillon culture, stop tubes with cotton, and stand upright in an incubator at 37° C. for one to two hours.

In a positive reaction the pool-serum tube should show no change, while the dilution tubes will show a granular deposit, which is easily distinguishable from a uniform turbidity shown in a negative reaction.

A simple method that is practised by many boards of health and in private laboratories is to dry a few drops of patient's blood on a piece of paper and transmit that to the laboratory for examination. Here the laboratory worker becomes accustomed to estimate the amount of blood forming the dried drop, and this is diluted with an equal amount of normal saline. After thorough softening of the drop, the whole is transferred by means of the loop to a watch-glass or slide. From this 50 per cent. dilution subsequent dilutions are made as described above.

The Serum Dilution.—It is usually held that a dilution of 1 to 40 or 1 to 50 with normal salt solution is sufficient to eliminate the possibility of false agglutination by normal sera, and at the same time sufficiently low to permit of the good reaction to nearly all typhoid cases, excepting possibly advanced convalescents.

If agglutination occurs in this preparation, we note, with the aid of the high power, that, in the course of from fifteen minutes to an hour as the micro-organisms swim about, a few as they come in contact have a tendency to remain in this relation. In the course of a few more minutes other organisms join this group and other groups form throughout the field; motility becomes gradually less until it ceases entirely in the typical reaction. The complete change occurs in from six to eight hours. *Not less than five bacteria must become permanently agglutinated to constitute a positive reaction.* The test is most

decisive when large masses of permanently agglutinated organisms are formed which can be seen with the lower power.

Method of Bass and Watkins.—These workers have introduced a modification of the macroscopic agglutination test, which is simple, reliable, and quick. It has the advantage that it can be carried out at the bedside, the result being known in two to three minutes. Little equipment is required. The suspension consists of 100 million killed typhoid organisms per cubic centimeter in 1.7 per cent. NaCl solution to which 1 per cent. formalin is added.

The Test.—Allow a full drop of blood to fall into 4 drops of water and mix thoroughly. Instead of this, one may make a blood-smear (using approximately ¼ drop of blood and dissolving this on the slide with 1 drop of water, the mixture being stirred with a tooth-pick or similar substance). With this diluted blood (1 to 4) mix an equal amount of the above suspension of typhoid bacilli on a glass slide. Tilt the slide from side to side so as to keep the mixture flowing back and forth. If the reaction is positive a grayish, mealy sediment appears within one minute, usually in less. This sediment appears in the fluid around the edges and tends to collect there. If the agitation is continued, the clumps increase in size for two to three minutes. If the reaction does not appear in this time, it will not appear at all. When the reaction is negative, no agglutination occurs and the mixture remains as clear and unchanged as when first put on the slide.

AGGLUTINATION REACTIONS IN DISEASES OTHER THAN TYPHOID.

Paratyphoid Infections.—A certain number of cases clinically resembling typhoid fever do not give an agglutination reaction with typhoid bacilli. From some of these cases a group of bacilli has been isolated, the members of which resemble typhoid bacilli in some of their cultural characteristics. They differ from the latter, however, especially in the formation of gas in glucose bouillon and by their serum reactions. The blood-serum of a patient suffering from infection with one of these varieties (paratyphoid) will agglutinate the species isolated from the patient's blood in the high dilutions; but not all cases

of paratyphoid fever will agglutinate equally well with paratyphoid bacilli isolated from different epidemics. There are now two well-recognized types of para-organisms. It is necessary, therefore, in case no agglutination is obtained with a typhoid bacillus, to secure members of both groups of the paratyphoid bacilli and to carry out agglutination tests with each. A high agglutination with any one of the varieties used indicates a probable infection with that variety of bacillus. The method of testing is similar to that described under Widal reaction.

Infections Due to the Bacillus Coli Communis.—General infections by the *Bacillus coli communis* show a moderate agglutinating power in the blood for fresh twenty-four-hour cultures of the organism.

Infections Due to Members of the Dysentery Group of Bacilli.—This group of bacilli contains at least three separate species, differing from each other in their agglutinations and reactions when grown upon sugar media. The bacillus originally isolated by Shiga[3] from cases of endemic dysentery in Japan has been shown to give a macroscopic agglutination with the blood of these patients in dilutions even as high as 1 to 40 or 1 to 60 in an hour.

The Flexner[4] type of bacillus has also been found to agglutinate in low dilutions with the blood of patients from whose stools this bacillus has been isolated.

Cholera.—A moderate number of tests have been carried out on the agglutinating reaction of serum from cholera patients on the specific bacillus, and such reactions have been found to appear at a fairly early period in the course of the disease, and develop in as high as 1 to 40 dilutions.

A diagnosis can be made earlier and more certainly by the isolation of the bacilli from the stools and testing them with an immune serum of high agglutinating power.[5]

Plague.—An agglutination of the plague bacillus has been observed beginning with the second week of the infection and rising later even as high as 1 to 40. The reaction, however, is of

[3] Deut. med. Wochen., 1901, p. 741.
[4] Bull. Johns Hopkins Hosp., 1900, vol. ii.
[5] Kolle and Gotschild: Zeit. f. Hygiene, 1903; Bd. xliv, p. 1.

little practical value, since the diagnosis can be made much earlier by the cultural isolation of the bacillus from the bubo or by the inoculation of susceptible animals.[6]

Malta Fever.—The serum of patients suffering from Malta fever usually gives a marked agglutination with the *Micrococcus melitensis*. The reaction has been seen to occur in a dilution as high as 1 to 50, or in some cases 1 to 300.[7] Using emulsions of dead bacteria in physiological saline solution, the agglutinating power of the serum may rise to 1 to 1000 or more, if the mixture is left for twenty-four hours at room temperature.[8]

Mandelbaum's Test for Typhoid Fever.[9]—Mandelbaum states that typhoid bacilli string out into long chains or the chains coil into a snarl when typhoid serum is added to a culture of the bacilli. This reaction seems to be specific, as he never observed it with any serum except that from typhoid patients. The reaction occurs in three or four hours and is readily perceptible in the hanging drop under the microscope by this time. This reaction may also occur long before the agglutination test gives positive findings. It also seems to occur with serum from persons with a history of typhoid in the past, although the reaction develops more slowly in such cases. The reaction was also slow but pronounced in a case of a chronic typhoid bacillus carrier. Mandelbaum has worked out a simple technic for the test, aiming to adapt it for general use. From 5 to 8 cubic centimeters of a solution of 2 grams of sodium citrate in 100 cubic centimeters of ordinary bouillon is sterilized and a portion placed in a test-tube, and then this sodium-citrate-bouillon tube is inoculated with a loop of a motile typhoid-bacillus-bouillon culture or a scrap from an agar culture of typhoid bacilli. The culture must not be recent, but capable of growing well when further cultivated. A drop of blood is then obtained from the patient, aspirated into a long tapering capillary pipette with rubber cap like a medicine dropper. Then ten or fifteen times as much of the sodium-citrate-bouillon inoculated with the typhoid-bacillus cultures is drawn up likewise into the pipette. By releasing the pressure on the rubber cap, the fluid gradually

[6] Martini: Zeit. f. Hygiene, 1902, Bd. xli, p. 159.
[7] Kretz: Wien. klin. Wochen., 1897, p. 1076.
[8] Basset-Smith: Brit. Med. Jour., 1902, p. 861.
[9] Münch. med. Wochen., Jan. 25, 1910, lvii, No. 4.

rises to fill the lower third of the broader part of the pipette. The tapering tip is then fused and the pipette well shaken to mix the contents. The pipette is then kept at a temperature of 37° C. (98.6° F.) for from three to four hours. The sodium citrate addition is for the purpose of preventing coagulation, and after this interval the red corpuscles will be found collected on the bottom of the pipette, with a clear fluid above. The rubber cap is then taken off and a drop of the clear fluid is taken for examination as a hanging drop under the microscope. If the blood came from a person with typhoid the bacilli in the hanging drop will be adherent, while with non-typhoid serum the bacilli are scattered through the fluid. When the chains are found, and also a few isolated bacilli between the chains and snarls, the reaction is typical of typhoid existent several years before. In the one chronic typhoid-bacillus carrier he was able to examine, the reaction at the fourth hour was typical of a positive existing typhoid, but then the picture gradually became modified, so that by the end of the eighth hour the hanging drop resembled the findings in an old cured case. According to his experience, there are thus three forms of the reaction, which, however, is not very extensive—only 12 cases of existing typhoid, 16 of long-past typhoid, and 1 typhoid-bacillus carrier. The findings were constant in each class and were constantly negative in 75 other persons apparently free from a history of typhoid. The pipettes are those used in opsonic tests.

Subsequent observations by W. Gaehtgens and W. Kamm[10] confirm the value of this test not only in the early stages of typhoid fever, but also in the detection of typhoid carriers.

This is a matter of great importance, as heretofore it has been almost impossible to identify these dangerous persons by any simple means, as in them the Widal is often of no value, while a certain detection could only be by cultural studies of an extensive character, carried out with specimens of urine and feces.

If the findings of these observers are further verified, this test of Mandelbaum will be a valuable addition to our laboratory technic.

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[10] Münch med. Wochen., 1910, lvii, Bd. 26.

THE PRINCIPLES OF THE WASSERMANN AND NOGUCHI REACTIONS, AND THEIR COMPARATIVE VALUE TO THE CLINICIAN.[11]

Since the appearance of the original communications of Wassermann, Neisser and Bruck announcing a new method of syphilitic diagnosis, medical literature both in Europe and in America has been literally flooded with papers dealing with this subject, and it is highly probable that no other single topic has received so much consideration from so many workers in the various fields of medicine. A very great deal of this work has been of the most careful and painstaking character. While the subject is a relatively narrow one the significance of a luetic infection is such that every aspect of the question of serum diagnosis has been subjected to the most careful scrutiny and has been investigated from both the clinical and experimental points of view, and now, after over three years, certain facts have been definitely determined.

It is perhaps as well at the outset to say, however, that neither of the reactions to be described can be regarded as absolutely specific.

The Wassermann reaction, based upon an ingenious principle, worked out by Bordet and Gengou, gives to the experienced laboratory worker a very satisfactory means of diagnosticating syphilitic affections, even in individuals who were infected years ago, of course excluding a dormant condition of patients at the end of a successful treatment.

In order to be able to grasp the steps in the Wassermann reaction it is indispensable to be acquainted with the steps involved in demonstrating antibodies of any kind formed accidentally (by disease) or purposely (immunizing) in the body fluids of an animal. Ehrlich showed long ago that in the demonstration of antibodies, or, as he called them, amboceptors, three distinctly different substances are required in order to form a complete reaction: First, the cell or poison against which we wish to immunize, or, more plainly, against which we desire to obtain an antibody; second, the antibody (or amboceptor) obtained by repeated injections of the special cell or poison into

[11] Kaplan: Am. Jour. of Med. Sci., Jan., 1910.

the rabbit (or any other suitable animal); third, a completing substance—the complement. This latter substance is present in variable quantities in the sera of all animals, its quantity being rather constant in guinea-pigs. It is destroyed by heating various sera to 56 or 57 degrees centigrade for one-half hour, and is similarly affected by various other physical agents. This is not the case with antibodies which are comparatively thermostable. We have then three factors: (1) A cell to be destroyed or a poison to be neutralized; (2) a substance capable of doing this—the amboceptor or antibody; and (3) a completing substance, without which the reaction cannot take place—the complement.

Ehrlich and others in order to impress the reaction upon the minds of men interested in immune processes, made use of diagrams. To make it still more familiar, let a lock represent the cell, a key which fits it the antibody, and the hand that will turn the key the complement. By giving a lock to a smith we can get a key made to fit the specific lock exactly. When we inject cells we can get an antibody which exactly fits the cell injected, and the same is true when we inject a bacillus or a poison. All these substances capable of producing antibodies (antibody generators) are known as antigens.

To determine whether a bacterium was killed or a poison neutralized by being exposed to the action of a specific amboceptor, is not as simple a process as the demonstration of the destruction of red blood-corpuscles by an amboceptor directed against them. A suspension of red blood-corpuscles minus amboceptor and complement gives an opaque red mixture; when we add the amboceptor plus complement and incubate at 37° C. the opacity disappears and a clear red fluid results. It is apparent that hemolysis or destruction of red cells is a phenomenon that can readily be seen *in vitro,* and its presence signifies that the three substances spoken of above are present in the test-tube. If any one of the three is not there, or is present in an inactive state, the red cells will remain unaffected and the mixture will retain an opaque, red color.

Hemolysis.—The phenomenon known as hemolysis depends upon the destruction of red blood-corpuscles. There are many reagents capable of doing this, such as distilled water, acids, and

alkalies. It is also possible to form in warm-blooded animals substances which will bring about hemolysis against certain red blood-cells. This is accomplished by injecting an animal (a rabbit or goat) with the cells of a sheep or any other animal. The serum from such a rabbit, when brought in contact with the cells of a sheep will cause the mixture to become clear (hemolysis). The same amount of serum from an untreated rabbit will have no effect on a similar suspension of sheep cells. The substance produced in the rabbit's serum is known as the anti-sheep amboceptor, and together with sheep cells and complement (from a guinea-pig) is known as a *hemolytic system.*

We learn from the above exposition that in order to prove the presence or absence of certain antibodies, we make use of the phenomenon of *bound* or *unbound complement,* utilizing a hemolytic system simply as an indicator. Exactly the same principle is applied to the serum diagnosis of syphilis.

Luetic Antigen.—Unable to produce a growth of spirochæte pallida upon any culture medium, we have to be contented with organic extracts containing them in greatest numbers, for this purpose the liver of the luetic fetus fills the requirement. The extract obtained is known as leutic antigen, and need not confuse anybody, for we know that an antigen is a body capable of forming antibodies. If an individual has had syphilis some years ago, he would also have syphilitic antibodies in his serum which, when brought in contact with the extract from the syphilitic liver, would invariably bind complement, and a hemolytic system (sheep cells or any other red cells with the corresponding amboceptors) will not be affected, because the complement had been bound previously.

Principle and Technic of the Wassermann Reaction.—As mentioned before, antibodies will attract complement if the antigen responsible for their formation is present in the same test-tube. In the Wassermann reaction a serum containing antibodies capable of uniting with the antigen is used (substance containing lipoids) and thus deviating the introduced complement, will not permit hemolysis to occur, if sheep cells and their antisheep amboceptor are subsequently placed in the same tube, as for obvious reasons, the complement was bound or deviated previously by conditions suitable for such an interaction. If

the patient's serum does not contain the required antibody, the introduced complement will remain unbound and in a fit condition to destroy the sheep cells when subsequently introduced with their antisheep amboceptor.

Modus Operandi.—(1) Obtaining blood from patient: A fairly stout piece of rubber tubing is placed a little above the elbow and held snugly in place by an artery clamp. Do not obliterate the pulse. This brings into prominence the veins at the bend of the elbow. To a stout hypodermic needle (use a 19 bore—one and one-half inch needle) attach a two-inch piece of rubber tubing. Holding the free end of the rubber tubing in an ordinary sterile test-tube, quickly plunge the needle into the most prominent vein; if expertly done, the patient will hardly feel it and the blood will immediately begin to flow. About 6 to 10 cubic centimeters of blood is withdrawn and placed in the ice box over night to coagulate. The serum separates and may be pipetted off absolutely clear without cells. It is advisable to take the blood as far from a meal as possible, as proximity to a meal makes the blood lipemic, interfering with perfect working conditions. (2) Having obtained 1 or 2 cubic centimeters of clear serum, it is placed in a test-tube in the thermostat at 56 degrees for one hour. Care must be taken not to permit the heat to rise too high (over 58 degrees). (3) After this, 0.2 cubic centimeter is placed in each of two test-tubes, one the test, the other the control. (4) To each is now added 0.1 cubic centimeter fresh complement. (5) To the test portion is added one unit of antigen. The control does not receive any antigen. (6) Each tube receives now 3 cubic centimeters of a 0.95 per cent. NaCl solution. In order to be able to judge properly the correctness of the procedure, the more controls one has the better; it is therefore necessary to compare the serum to be tested with two sera from known positive and negative bloods. (7) Shake every tube well and place in incubator at 37 or 38 degrees for one hour. During this time, if the serum is luetic the antibodies present will, together with the antigen, bind the complement and render it inactive for hemolysis. (8) After one hour incubation each tube receives two units of amboceptor and 1 cubic centimeter of a 5 per cent. suspension of sheep cells in 0.95 NaCl. The tubes are again vigorously

shaken and placed in the incubator at 37 degrees and inspected after ten minutes. If the reagents are properly adjusted hemolysis begins in the control tubes in fifteen to twenty minutes, and careful watching becomes a very essential point at this stage of the test. As soon as the control is completely hemolyzed the tubes are to be compared; only those should be pronounced negative that show a transparent fluid the same as the control.

Permitting the tubes to stand undisturbed in a cool place (15 to 17 degrees C.) for twenty-four hours shows in the positive test a deposit of red cells, the size of the deposit depending upon the severity of the infection or proximity to the initial lesion as well as upon the degree of balance of the reagents used. Usually a markedly positive serum gives at the end of twenty-four hours a clear supernatant fluid of a light pink hue with a Bordeaux red accumulation on the bottom of the tube. The weaker the reaction, the redder the supernatant fluid and the scantier the deposit of cells. In testing more than one serum, the reaction in each individual test must be considered as finished as soon as the controls are completely hemolyzed, in which case the two tubes are immediately removed to a cool place.

Principle and Technic of the Noguchi Reaction.—This is same as in the Wassermann, excepting that the amboceptor is directed against human cells. It also facilitates the handling of reagents, as they are mostly paper soaked in the antigen and amboceptor. These do not readily deteriorate, as is the case with fluid biological reagents. The serum does not need inactivation at 56° C.

Modus Operandi.—(1) With a capillary pipette allow one drop of fresh serum to fall into a narrow (1 centimeter lumen) test-tube. The pipette is not to be used for any other serum. (2) Add 0.05 cubic centimeter fresh complement. (3) To the front row (rear row for control) add one piece (more or less, depending on the titre) of antigen paper. (4) Prepare a suspension of human cells 1 drop of blood to 4 cubic centimeters NaCl 0.95 per cent. It is best to prepare about 60 cubic centimeters of NaCl solution and allow 15 drops of blood to fall from the experimenter's finger into the solution. The human cell suspension is placed over night in the ice box. Next morning the supernatant clear salt solution is pipetted off and a fresh

quantity of NaCl is added (about 55 cubic centimeters) to the cells in the beaker. Of this cell suspension add 1 cubic centimeter to every tube in the rack. (5) Incubate for three-quarters or one hour at 38 or 39 degrees, preferably in a large dish of warm water. Occasionally shake the tubes, to insure proper solution of the biological substance on the antigen paper. (6) Add to each tube (after incubation), front and back rows, one piece of amboceptor paper more or less, the quantity depending on the titre) and replace in the incubator, observing the result after ten minutes, and watching carefully the controls.

It will be noted in about fifteen minutes, more or less, that the rear row begins to get clear, and when complete transparency is obtained the test and control tube are to be removed to a cool place and observed. If the reaction is positive, then the front tube (test) will be opaque, in marked contrast to the control, which is transparent. For convenience of observation, make use of a fine sealed tube (about 1 millimeter in diameter) filled with black ink, which, when placed behind the control, will appear as a clear black line, whereas the positive tube will not show the black line, or it appears as a dim shadow—depending on the strength of the reaction.

It has been stated that a positive Noguchi test and a negative Wassermann is often due to the presence in the patient's serum of antisheep amboceptors. It is not necessary to perform this test with every serum as a control. Only sera giving the above results need be subjected to a verification. To demonstrate the antisheep amboceptor, place 1 cubic centimeter of a 5 per cent. suspension of sheep cells in a test-tube, add 0.2 cubic centimeter of patient's serum and 0.1 cubic centimeter complement, add 3 cubic centimeters of NaCl solution, place in incubator, and observe. If the amboceptor is present, the cells will dissolve and the mixture become transparent. The time consumed depends upon the number of amboceptor units present.

Controls.—In the Wassermann and Noguchi reactions it is of vital importance to have every possible error excluded. The substances to be controlled are the antigen, the amboceptor, and each individual serum.

The Antigen Control.—This biological reagent, as is known, can *per se* inhibit hemolysis. To measure the degree of such

interference, a tube containing a well-known normal serum, plus antigen, plus complement, and antisheep amboceptor plus sheep cells ought to hemolyze in about twenty to thirty minutes. No reaction is to be considered as finished before the antigen control tube is completely hemolyzed.

The Amboceptor Control.—Upon the efficiency of the antisheep amboceptor depends the rapidity of hemolysis of the sheep cells. It is therefore necessary to establish the amboceptor efficiency in a separate tube containing sheep cells, plus complement, plus antisheep amboceptor. It is not essential to add normal serum. The tube containing the above ingredients is always the first to hemolyze, requiring about fifteen to twenty minutes for a complete hemolysis.

Control for Each Serum.—Every serum more or less has the power to interfere with hemolysis to a slight degree. In order to control the factor of individual inhibition, every serum tested is placed in each of two tubes, the front tube contains the antigen and all other biological reagents, the rear tube receives everything but the antigen. This shows the degree of individual inhibition as compared with the tube containing the amboceptor control.

Efficiency of the Entire System.—For this a well-known luetic serum is utilized. The reaction is to be positive, and hemolysis should not occur in the front tube, even if exposed to incubation temperature for hours after the controls hemolyzed.

Equipment.—At least one dozen or even more of Mohr's pipettes, 1 cubic centimeter, graduated into $\frac{1}{100}$. One dozen 10 cubic centimeter pipettes, graduated into $\frac{1}{10}$. One gross of ordinary test-tubes. One gross of test-tubes 1 centimeter in diameter, 12 centimeters high. One-quarter dozen of graduated cylinders, 50 cubic centimeters; one-quarter dozen 100 cubic centimeters. Two 50 cubic centimeters measuring flasks with glass stoppers. A few pounds of glass tubing, 5 millimeters bore, to make capillary pipettes. One-half dozen test-tube racks for Wassermann tubes; one-half dozen test-tube racks for Noguchi tubes. A piece of rubber tubing for tourniquet. One artery clamp for above. One dozen hypodermic needles, 19 bore. One thermostat regulated at 57 degrees and one regulated at 37 degrees. One electric centrifuge. Labels and pencil for writing

on glass. One tall glass jar for flushing through used pipettes, height to be greater than any pipette used. One dozen Petri dishes. One dozen beakers, 100 cubic centimeters capacity. Two fine forceps, and two Hagedorn needles. One package of quantitative filter paper. One razor (for killing guinea-pigs). One 15 cubic centimeter Luer syringe.

Preparation of Animals. — *Antisheep Amboceptors:* Several healthy rabbits (not less than four) receive every fifth day 1, 2, 3, 4 and 6 cubic centimeters of well-washed sheep cells. This number of rabbits is used, as one or two may die during the injection weeks. The cells are obtained from the slaughter house and immediately defibrinated with a wire defibrinator or glass beads. In order thoroughly to wash the cells a high speed centrifuge is necessary, capable of making at least 3000 revolutions to the minute. Two of the centrifuge tubes are filled with the fluid sheep blood, it being advisable, in order not to spoil the centrifuge, to have them of equal weight. The first centrifugalization brings the cells to the bottom, and the clear supernatant serum is pipetted off. The cells are now mixed with 0.95 per cent. NaCl solution and centrifugalized again, and the supernatant clear fluid is again pipetted off; this is repeated three times. The cells are now approximately serum-free. The entire quantity of cells in the tube is now brought up to its original volume with 0.95 per cent. NaCl solution, and of this, 2 cubic centimeters is used for the first injection. With a sterile glass syringe this quantity is injected into the peritoneal cavity, having previously shaved and cleaned the puncture area. Cotton and collodion prevent wound infection. This procedure is repeated five days later with 4 cubic centimeters of cells brought to its original volume, etc., until each animal has been injected five times. Nine days having elapsed since the fifth injection, the serum of the rabbit contains now a high lytic power against the red blood-corpuscles of the sheep. The rabbit is killed and its serum used.

In the Wassermann reaction 1 cubic centimeter of a 5 per cent. suspension of well-washed sheep red cells in 0.95 per cent. NaCl solution is the standard dose for each test. It is evident, therefore, that in order to test the power of our rabbit serum (antisheep amboceptor, as it is now called) we must use this

quantity of sheep cells. Into each of six test-tubes is placed 1 cubic centimeter of a 5 per cent. suspension of sheep cells in NaCl 0.95 per cent. These are marked from 1 to 6, and to each is added 0.1 cubic centimeter of fresh guinea-pig serum (this is known as the complement serum and is the quantity used in the Wassermann test). We now add to test-tube (1) 1 cubic centimeter of a 1 to 200 solution of our amboceptor. To test-tube (2) we add 1 cubic centimeter of 1 to 400; to test-tube (3), 1 cubic centimeter of 1 to 800; to test-tube (4), 1 cubic centimeter of 1 to 1600; to test-tube (5), 1 cubic centimeter of 1 to 3200. This is placed into the thermostat at 37 degrees and the result noticed after fifteen minutes, thirty minutes, up to two hours. It will be seen that in fifteen minutes test-tube (1) is clearing up, or is clear (hemolysis); this would indicate that 1 cubic centimeter of a 1 to 200 solution of our amboceptor is capable of destroying in fifteen minutes 1 cubic centimeter of a 5 per cent. suspension of sheep cells. This proportion—1 to 200—is too strong, and may give negative results with some positive sera. The unit strength of the antisheep amboceptor is usually twice the quantity capable of hemolyzing the 1 cubic centimeter of cells in two hours. If 1 to 1600 shows hemolysis after two hours and 1 to 3200 does not, then 1600 divided by 2 is the strength of the amboceptor, one unit equals 1 to 800. It is best to run two series of titration—one like the above, the second beginning with 1 to 250, 1 to 500, 1 to 1000, etc., so that a proper mean can be established and a more exact unit made. In table form the above is expressed as follows:—

		Sheep cells.	Cells hemolyzed in
Tube 1 amboceptor = 1 to 200	Complement 0.1	5% 1 c.c.	15 minutes
" 2 " = 1 to 400	" 0.1	" "	30 minutes
" 3 " = 1 to 800	" 0.1	" "	50 minutes
" 4 " = 1 to 1600	" 0.1	" "	1½ hours
" 5 " = 1 to 3200	" 0.1	" "	2 hours

Strength of 1 unit, 1 to 1600; dose for 1 test, 1 to 800. Date.....

A full-grown rabbit usually furnishes from 50 to 60 cubic centimeters of serum. This is to be kept in a glass-stoppered flask in the ice box (lower compartment). The hemolytic power does not indefinitely remain the same as in the beginning; it is,

therefore, necessary to establish the titre at least once every week, and to make up the dilutions accordingly. These dilutions are to be prepared on the day of testing. The rabbit serum does not have to be inactivated to get rid of the complement in it, as the quantity of serum used is too small to influence in any way the resulting outcome of the test.

Preparation of Complement.—A full-grown guinea-pig is held over a Petri dish, and after having it narcotized, the blood-vessels of the neck are severed with a razor. Suspended by the hind legs the animal is exsanguinated, and the collected blood is permitted to remain at room temperature for at least three hours. The serum collects in large drops and may be pipetted off, or the coagulum plus the serum is placed in a centrifuge tube and after five minutes centrifugalization the supernatant serum is pipetted off into a sterile test-tube; but such a serum is not as reliable as when left for three hours with its cells. About 6 cubic centimeters of complement is obtained from one guinea-pig.

Preparation of Sheep Cells.—Obtained from the slaughter house, the cells are washed three times with 0.95 per cent. NaCl solution, and 1 or 2 cubic centimeters is mixed with 20 or 40 cubic centimeters of salt solution, making a 5 per cent. suspension of cells.

Preparation of Antigen.—The fresh liver of a luetic fetus or the liver of any baby cadaver is chopped up very finely, and the mass is spread on a few Petri dishes and dried. The drying process is hastened by a current of air produced by an ordinary electric fan. Lately, not only baby livers, but also the livers of dogs, the hearts of guinea-pigs, and other organs were used to make antigen. The usefulness of the antigen is only established when in actual standardization it is found serviceable and works faultlessly with decidedly syphilitic and unquestionably normal sera. Consequently it makes little difference whether one uses the extract obtained from the liver of a syphilitic fetus or from the heart of a guinea-pig, provided they are well titrated.

It is better—according to German workers—to use more than one extract, and have a series with well standardized luetic liver antigen, one with guinea-pig heart, and another with dog liver, or normal human liver. To proceed with the making of

antigen the obtained dried liver is rubbed into a powder and kept in an exsiccator over $CaCl_2$ in a cool, dark place. According to Tschernogubow, such a powder is serviceable for a very long time. Of this powder, 0.5 gram is extracted at room temperature or in an ice box with 25 cubic centimeters of 95 per cent. alcohol for twenty hours, then filtered, and the filtrate used for experiments.

For the actual Wassermann test, one part of this opalescent filtrate is diluted with five parts of 0.95 per cent. NaCl, and 0.5 to 1 cubic centimeter used for each test-tube, the dose depending upon the established titre. The above process extracts from the liver substances soluble in alcohol, chiefly bodies of a fatty nature (lipoids). There are other means of obtaining lipoids, the above being one of the simplest, having also in view the preservation of the antigen in an active form. The liver, instead of being dried and powdered, may be directly extracted with five volumes of absolute alcohol, and the extract obtained by driving the alcohol off at a temperature not higher than 40° C. or with the electric fan. The obtained extract is much more powerful than the above, is soluble in ether, from which NaCl solutions are made for use. The titre is established carefully as follows:

Titration of Antigen.—The unit dose of antigen must be of such a strength that one unit will completely inhibit hemolysis of 1 cubic centimeter of a 5 per cent. suspension of sheep cells, with 0.2 cubic centimeters of a known luetic serum plus 0.1 cubic centimeter of complement; provided double this dose does not interfere with the complete hemolysis of cells using a known normal serum and complement.

TABLE OF ANTIGEN STANDARDIZATION.

Luetic Series		*Normal Series*	
Each tube contains syphilitic serum, 0.2; complement, 0.1; cells, 1 c.c. 5%; ambocepter, 2 units.		Each tube contains normal serum, 0.2, complement, 0.1; cells, 1 c.c., 5%; amboceptor, 2 units.	
			Hemolysis
Tube 1, Antigen, 0.025	1 hour 15 min.	Antigen, 0.025	15 minutes
" 2, " 0.05	1 " 25 "	" 0.05	15 "
" 3, " 0.075	2 " 50 "	" 0.75	15 "
" 4, " 0.10 }	No hemolysis af-	" 0.10	20 "
" 5, " 0.15 }	ter 24 hours in	" 0.15	30 "
" 6, " 0.20 }	incubator.	" 0.20	35 "

Dose of 1 unit 0.1 cc.

From the above facts it is evident that the dose next to the largest hemolyzing dose is the strength of one unit, or 0.1 cubic centimeter. It is also apparent that 0.2 cubic centimeter, or a double dose, will not inhibit hemolysis when used with a normal serum.

In establishing the unit dose of antigen as well as antisheep amboceptor it is of utmost importance to titrate two or three times in order to get as uniform results as possible, and only uniform work will enable one to come to a proper conclusion as to which is the necessary dose. For establishing the strength of the antigen and amboceptor, well-known fresh luetic and normal sera are to be used, as well as fresh suspension of cells and fresh complement.

Before using the standardized reagents it is advisable to perform two or three actual tests with well-known positive and negative sera. After this the substances may be considered safe for use. The above lines will give one a fair idea concerning the preparation of the biological reagents for the Wassermann reaction. For the Noguchi reaction it will be necessary, first, to acquaint the reader with the principles involved, and then the preparation of reagents will follow.

Preparation of Reagents. — *Antihuman Amboceptor*: Rabbits are injected with human cells the same as the sheep rabbits. After nine days the rabbits are killed, their serum collected and disposed of as follows: The fluid amboceptor loses strength on standing, so much so that it may not contain one-fourth of its original power a month after the first titration. In a dry state it can be used for a very long time without losing its strength. Prepare antihuman amboceptor by cutting quantitative filter paper in 5 millimeter squares. These squares are stuck onto pins fastened to a cardboard. With a very fine capillary pipette (as fine a one as can be made) one drop is blown on each piece of filter paper and placed in the thermostat for drying. In half an hour the papers are dry and fit for use. By this method each square receives exactly the same quantity of serum, and is not subject to differences in dissemination which must be considered when the serum is blown on a larger piece of filter paper and cut subsequently in 5 millimeter squares. The method takes longer, but the difference is worth while, for each

square holds exactly the same quantity of amboceptor. In the test one piece of this amboceptor is serviceable.

TITRATION OF ANTIHUMAN AMBOCEPTORS.

	Human cells 1 drop to 4 c.c. NaCl.	Complement.	Hemolysis in
Tube 1, Amboceptor, ½ piece	1 c.c.	0.05	2 hours.
" 2, " 1 "	1 c.c.	0.05	20 "
" 3, " 1½ "	1 c.c.	0.05	11 "
" 4, " 2 "	1 c.c.	0.05	8 "

VALUE OF THE NOGUCHI TEST.—Workers with the Wassermann reaction often could not explain why a positive result could not be obtained with some true luetic sera. Later it was demonstrated that this was due to the presence in the human serum of substances capable of dissolving the red blood-corpuscles of the sheep; in other words, some human sera contained anti-sheep amboceptors.

Sometimes the quantity of antibody is so small that a goodly portion of the complement escapes unbound and does its work by bringing about partial hemolysis; that is the condition of affairs taking place in some weak reactions. If such a serum contained antisheep amboceptors, they would have enough complement to cause hemolysis and render the result negative.

In the Noguchi test this cannot take place, for the hemolytic system used consists of human cells plus antihuman amboceptor, and, naturally, the human organism does not contain antihuman amboceptors. Luetic sera containing antisheep amboceptors will give a negative Wassermann but a positive Noguchi test.

As a result of much work with the Wassermann and Noguchi tests, Kaplan is able to give a fair opinion as to its uses. Shortly stated, the two reactions are of the foremost importance to the clinician, and so far as accuracy is concerned, they almost occupy the first place among our means of detecting diseases.

The Wassermann reaction gives a negative result in 8 or 9 per cent. of syphilitic sera. This rather undesirably high percentage of error is reduced to 1 to 1.5 per cent. when using the Noguchi and the Wassermann combined.

Never render a decision after one test. Always perform two Wassermann and two Noguchi tests on different days, using the same serum. It is also to be borne in mind that a fairly marked Wassermann reaction, 99 times out of 100, means syphilis, and that a negative Noguchi the same number of times means no syphilis. The two methods are very decisive, but in opposite ways; and used together, carry with them an assurance which no amount of thoroughness and precision will replace if only one method is used.

The laboratory worker, being responsible to the clinician for his statements, ought to be in a position to help him considerably, but only when his work has been carried out very carefully, unbiased by personal opinion, and submitting the result of a delicate test as read from the test-tube.

It is advisable to work with both methods, the Wassermann and Noguchi, as one is a check on the other, which, if properly performed, should give correct reports in 98 per cent. of cases.

Questionable reactions are not to be used for diagnosis, and if a serum does not react strongly after a number of repetitions of the test, the diagnosis is to be left to the clinician.

Exceptionally strong reactions are obtained in untreated cases of general paresis with both tests, as well as in primary sores four weeks after infection.

Luetin Reaction.—Noguchi first announced his cutaneous reaction for syphilis in 1911.[12] Its chief value lies in the fact that it aids in the diagnosis of this condition as soon as the infection has gained entrance into the system and often antedates the occurrence of the Wassermann reaction. His material is prepared as follows: Pure cultures of the *Treponema pallidum* (see page 142) are allowed to grow for periods of six, twelve, twenty-four, and fifty days at 37° C. under anaërobic conditions. They may be cultivated in ascitic fluid containing a piece of sterile placenta, or in ascitic fluid agar also containing placenta. The lower portion of each solid culture is cut out and the tissue removed. These agar columns, containing large numbers of spirochetes, are carefully ground in a sterile motor. This paste is then gradually diluted by adding, little by little, the fluid culture, until the emulsion becomes perfectly liquid.

[12] Jour. of Exp. Med., vol. xiv, 1911, p. 557.

This mixture is heated to 60° C. for one hour in a water-bath and 0.5 per cent. carbolic acid is added. When this mixture is examined under the dark-field microscope 40 to 100 pallidæ may be seen in each field. This suspension is called *Luetin.*

Principle of the Test.—The skin of animals repeatedly inoculated with *Spirochæta pallida* and the skin of human beings suffering from syphilis do not behave in the same way as does the skin of non-syphilitic individuals. The skin of the syphilitic subjects reacts with an inflammation to the inoculation of the pallida substances (the luetin) prepared from the killed pure cultures, while the non-syphilitic skin does not. The intensity of the inflammatory reaction produced by the luetin may vary from an inflammatory nodule to a pustule formation, lasting, as a rule, for several days. In some instances the reaction may commence as late as three or four weeks after the inoculation.

Technic of Application.—The skin of the upper arm is sterilized with alcoholic sublimate solution before the injection. The amount of luetin injected is 0.05 cubic centimeter. This injection is intradermic, that is, in the skin, as superficially as possible. In non-infected cases there appears, after twenty-four hours, a very small, erythematous area at and around the point of injection. No pain or itching is experienced. This slight reaction gradually recedes within forty-eight hours and leaves no induration. In certain cases the reaction may reach a stage of small papule formation after twenty-four or forty-eight hours, after which time it commences to recede.

The Reaction.—In positive cases the following types of reaction occur:—

(*a*) *Papular Form.*—A large, raised, reddish, indurated papule, usually 5 to 10 millimeters in diameter, makes its appearance in twenty-four to forty-eight hours. The papule may be surrounded by a diffuse zone of redness and show marked telangiectasis. The dimensions and the degrees of induration slowly increase during the following three or four days, after which the inflammatory processes begin to recede. The color of the papule gradually becomes dark bluish red.

(*b*) *Pustular Form.*—The beginning and course of this reaction resemble the papular form until about the fourth or fifth day, when the inflammatory processes commence to pro-

gress. The surface of the indurated round papule becomes mildly edematous, and multiple miliary vesicles occasionally form. At the same time a beginning central softening of the papule obtains. Within the next twenty-four hours, the papule changes into a vesicle, filled at first with a semiopaque serum, that later becomes definitely purulent. Soon the pustule ruptures.

(*c*) *Torpid Form.*—In rare instances the injection sites fade away to almost invisible points within three or four days, so that they may be passed over as negative reactions. Sometimes these spots suddenly light up again after ten days or so and progress to small pustular formation.

No marked constitutional symptoms have been observed after the use of the luetin. In most positive cases a slight rise in temperature takes place, lasting for one day.

Specificity.—Speaking summarily (Noguchi), the following facts have been established:—

1. The luetin reaction is specific for syphilis.

2. The reaction is present in the majority of cases of tertiary, latent, and hereditary syphilis.

3. It is less constantly present in secondary untreated and primary cases.

4. In treated secondary cases the reaction is present in most instances.

5. In general paralysis and tabes dorsalis the reaction is inconstant, but a positive reaction was obtained by Moore and Noguchi in about 60 per cent. of cases.

6. In certain cases of tertiary and hereditary syphilis there may be a considerable inflammatory reaction at the site of injection of the control fluid, and the reaction may sometimes be as strong as that produced at the luetin inoculation site.

7. The condition of the skin which gives the luetin reaction remains but little influenced by the antisyphilitic treatment, although a positive reaction can no longer be obtained in some cases which had been thoroughly treated and believed to be cured.

The relation between the Wassermann and the luetin reactions may be defined here in order that the fullest possible benefit may be derived from the use of both reactions. The Wassermann reaction is more constantly present than the luetin

reaction in cases of primary and secondary syphilis, especially when only a slight amount of treatment or none has been given. On the other hand, the luetin reaction is more constantly present than the Wassermann reaction in cases of tertiary and latent syphilis. Besides, in cases in which the Wassermann reaction and clinical manifestations of syphilis are very marked and the luetin reaction negative, an energetic treatment can reverse the situation completely.

Thus, through the treatment the Wassermann and clinical symptoms gradually are made to disappear, while the luetin reaction becomes more distinct and the condition that gives the reaction to the luetin persists afterward, probably until a cure is effected. From the above-cited facts it may be concluded that the luetin reaction possesses greater diagnostic value than the Wassermann in tertiary and latent syphilis, and also a decided prognostic value which the Wassermann does not.

Caution.—While the application of the luetin reaction is simple enough to be within the reach of any physician, one must not underestimate the technical precautions necessary for obtaining reliable results, because all kinds of irregularity of the reaction can be obtained by a neglectful and faulty technic. Besides, it is important to become quite familiar with the reactions before one can recognize the milder form of positive reaction and avoid a misinterpretation.

A NEW TEST FOR PREGNANCY.

Abderhalden's Anaphylactic Reaction.[13]—The study of anaphylaxis has shown that whenever a foreign proteid is introduced into the circulation the tissues react, with the production of a ferment possessing the power of disintegrating this proteid. The proteids of the placenta, especially of the chorion, act as foreign proteids and cause the appearance, in the blood of the pregnant woman, of ferments able to disintegrate them. If placental extract is mixed with ordinary blood-serum, no change results. If the blood-serum was, however, obtained from a pregnant woman, the placental proteids are disintegrated, with the formation of peptones.

[13] Münch. med. Wochen., No. 24, 1912.

The test, which may become of great practical importance, is done as follows: A mixture of human placental extract and the serum to be tested are placed in a dialyzing tube, the latter being immersed in distilled water. The appearance of a biuret reaction in the water indicates that the serum was obtained from a pregnant woman. The full technic of this reaction is rather tedious and exacting, although not presenting any great technical difficulties, the elaboration of which will be found in the reference given above and in New York Med. Jour., vol. xcix, 1914.

Meiostagmin Reaction.—G. Izar[14] and Ascoli call attention to a specific test which is named the meiostagmin reaction. Izar measures the size of the drop with a stalagmometer (an instrument for measuring the size of drops), and announces that the drop-forming property of various fluids becomes modified in certain pathologic conditions. Izar has applied the test to syphilitic sera and found that the addition of the syphilis antigen increased the number of drops in the test fluid, while the serum of non-syphilitics had no such influence.

Technic.—Use for the antigen an alcoholic extract of the spleen of a syphilitic fetus (0.5 gram of pulverized spleen mixed with 50 cubic centimeters of alcohol incubated for two hours, filtered and evaporated to 10 cubic centimeters, which was then diluted 1 to 100 parts with 0.85 per cent. salt solution). The blood-serum is diluted 1 to 100 with similar solution. The number of drops formed by the diluted serum is determined before addition of the antigen. Then 1 cubic centimeter of the diluted serum is tested in the same way before adding the antigen. Then 1 cubic centimeter of the diluted antigen is added to 9 cubic centimeters of the diluted serum, and the whole kept at 37° C. (98.6° F.) for two hours, before the number of drops is again determined. There were always from 2 to 5 more drops in the fluid after addition of the antigen. Two investigations of serum from patients with leprosy gave no change in the number of drops. This test is applicable to typhoid fever. Admixture of typhoid serum with an extract of typhoid bacilli increases the number of drops which the fluid is able to form. A positive specific reaction has also been obtained in tuberculosis.

[14] Münch. med. Wochen., Jan. 25, 1910, lvii, No. 4.

Opsonic Method.—Since the publication of Wright's original researches, the unknown factors of bodily resistance and the development of clinical methods, whereby the bactericidal power of the blood, including its several components, have been carefully studied. Wright, Douglass, and Reid, continuing the study as well as others, have done an immense amount of research and clinical work along these lines. The universal employment of bacterial vaccine treatment has demonstrated the fact that with rare exceptions the laboratory estimation of the opsonic index is superfluous, and that in the large majority of cases a perfectly satisfactory result may be obtained by what may be termed the routine spacing of the intervals between doses.

For this reason and because of the technical difficulty surrounding an estimation of the opsonic index, the author believes it unnecessary to go into this technic in detail or to further discuss its use or value here. Current literature has many references to it, and a fair number of works on diagnostic and bacteriologic methods furnish a reliable guide for those desiring to employ it.

XIII.

APPENDIX.

DESCRIPTION OF OFFICE LABORATORY CABINET.

Fig. 56 shows a semi-portable laboratory cabinet containing reagents and apparatus sufficient to perform a majority of the

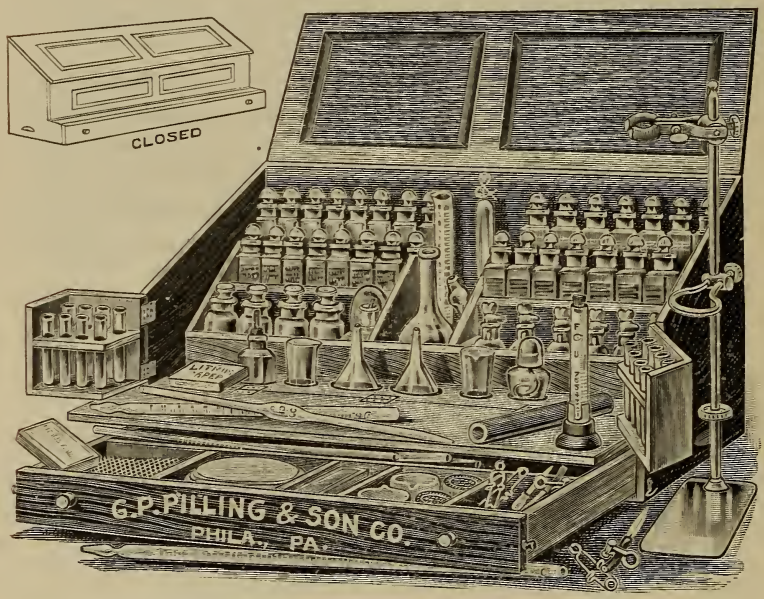


Fig. 56.—Portable Clinical Laboratory Set, after Plan Suggested by the Author. (G. P. P. & Son Co.)

simpler tests employed in the examination of uncertain sputum, gastric contents, and feces; also for the preparation of microscopic specimens of the blood and of bacteria.

This may be obtained in the open market and will be found of great service to those desiring to follow clinical methods in connection with the study of their cases. It is finished in quartered oak. The dimensions are approximately 13 by 15 by 26 inches.

CLINICAL TERMS.

Owing to the various and uncertain terms employed to designate the amounts of substances found, in clinical investigations the following scheme has been successfully employed in our laboratory.

Terms to be used in expressing the results of examinations of specimens in laboratory[1]:—

Albumin, Sugar, Indican, Acetone, Bile, Blood, etc.	Questionable trace.
	Very faint trace.
	Faint trace
	Trace.
	Strong trace.
	Moderate amount.
	Large amount.
	Very large amount.
	Excessively large amount.

For Sediments use:—

Occasional.

Few.

Moderate number.

Many.

Very many.

Excessively large number.

[1] After Judson Daland.

APPARATUS.

The following apparatus will be found necessary to conduct the examinations described in this book:—

FOR URINALYSIS.

Pinch-cock.
Pipette, 1 c.c.
Pipette, 5 c.c.
Pipette, 10 c.c.
Flask, 1000 c.c.
Flask, 100 c.c.
Graduated cylinder, 50 c.c.
Urinometer and jar.
Ureometer, Doremus.
Albuminometer, Esbach.
Plain glass pipettes.
Glass stirring-rod.
Porcelain dishes.
Porcelain tile.
Beakers, 30, 100, and 300 c.c.
Test-tube rack.
Ruheman's uricometer.
Sedimentation glasses.
Cover-glasses, Nos. 1 and 2.
Funnels.
Test-tubes, assorted sizes.
Centrifuge.
Plain centrifuge tubes.
Graduated Purdy tubes.
Bunsen burner and hose.
Tripod.
Wire gauze.
Burette stand.
Filter paper.
Microscope.
Westphal balance.
Forceps (cover-glass and large)
Glass tubing.

FOR BLOOD AND BLOOD-PRESSURE.

Gowers's, Fleischl's, or Sahli's hemoglobinometer.
Thoma-Zeiss hemocytometer.
Bottles with dropping-stoppers.
Hypodermic syringe.
Faught sphygmomanometer.
Daland lancet.
Test-tube brush.
Microscope slides.
Mechanical stage.
Spectroscope.
Dorrance's coagulometer or Bogg's modification of Russell and Brodie's apparatus.

FOR STOMACH EXAMINATION.

Two Mohr's burettes (25 and 50 cubic centimeters) will be required.

FOR FECES, CEREBROSPINAL FLUID AND MILK.

Stool sieve.
Breast-pump.
Hydrometer, 1010 to 1040.
Graduated cream-gauge.
Cream centrifuge tube and pipettes.
Capillary tube, heavy glass. Diameter, 0.05 millimeter. Of varying length.

FOR BACTERIOLOGIC AND OPSONIC WORK.

Incubator.
1 pr. balances accurate to 0.2 gram.
Bunsen burner.
Thermometer registering 200° C.
Thermometer graduated in tenths. Registering from 0° to 50° C.
Large watch-crystals.
Pure culture of typhoid bacillus.
Arnold steam sterilizer.
Thermo-regulator.
Rubber tubing.
Platinum wire loops, glass handle.
Sterile bouillon in tubes.
Agar slants.
Capillary pipettes.
Hanging drop slides.
Labels.
Petri dishes.

CHEMICALS AND REAGENTS.

The following chemicals and reagents will be required to carry out the examinations outlined in this work:—

CHEMICALS—FOR URINALYSIS.

Acid, nitric.
" sulphuric.
" hydrochloric, C. P.
" hydrochloric, deci-normal.
" acetic, glacial.
" acetic, 10 per cent.
" salicylic.
Ammonium hydrate.
Potassium hydrate (sticks).
" " 20 per cent. solution.
Sodium hydrate (sticks).
" " 40 per cent. solution.
" " deci-normal solution.
Silver nitrate, standard sol.

CHEMICALS—FOR URINALYSIS (*continued*).

Copper sulphate, 1 to 10 sol.
Ammonium sulphate, sat. sol.
Sodium chlorid, sat. sol.
Litmus papers.
Ethyl alcohol.
Methyl alcohol.
Amylic alcohol.
Potassium chlorate, 1 per cent. sol.
Lead acetate (tribasic).
Sodium acetate.
Potassium ferrocyanid.
Zinc chlorid, 5 per cent. aqu. sol.
Potassium acetate.
Chloral.
Sodium carbonate.
Neutral ferric chlorid.
Barium chlorid, standard sol.
Sodium nitroprussid.
Solution hydrogen dioxid.
Bromine water.
Phenylhydrazin hydrochlorid.
Ethylene diamine hydrate, 10 per cent. aqueous.
Zinc acetate, 10 per cent. alcoholic sol.
Phenolphthalein, 1 per cent. alcohol sol.
Powdered guaiac.
Orcin.
Chloroform.
Lead carbonate.

FOR BLOOD.

Normal saline solution.
Benzol.
Xylol.
Chloroform.
Litmus paper, red and blue.
Canada balsam.
Xylol balsam.
Xylol dammar.
Ether.
½ per cent. acetic acid.
2½ per cent. potassium bichromate.
Dimethyl-amido-azobenzol paper.
Dimethyl-amido-azobenzol (Töpfer's reagent).
Deci-normal sodium hydrate.
Phenolphthalein (1 per cent. alcoholic).
Sol. neutral ferric chlorid.
Congo-red paper.
Starch paper.
Powdered pepsin.
Sodium carbonate.
Potassium hydrate, 10 per cent. sol.
Egg albumin in cubes or buttons preserved in glycerin.
Mett's capillary albumin tubes.

REAGENTS—FOR URINE.

MAGNESIA MIXTURE:—

Ammonium chlorid	1 part
Magnesium sulphate	1 part
Ammonia water	1 part
Water	8 parts

The salts are dissolved in the water and the ammonia water then added.

KNOP'S SOLUTION:—

Bottle "A"—Sodium hydrate sol.	1:25
Bottle "B"—Bromine	1 part.
KBr	1 part.
Water	8 parts.

For the test add 1 part of the bromine solution to 15 or 20 parts of the sodium hydrate solution.

PURDY'S REAGENT:—

Potassium ferrocyanid	10 parts.
Strong acetic acid	10 parts.
Water	10 parts.

TANRET'S REAGENT:—

Dissolve 33.1 grams potassium iodin in 200 cubic centimeters of water. Add 13.5 grams powdered mercuric chlorid and water, stirring until the red precipitate first formed has been dissolved.

Dilute to 900 cubic centimeters with water and add 100 cubic centimeters strong acetic acid. Allow to stand twelve hours, and then decant from precipitate and use clear solution. This forms a solution of mercuric-potassium iodid in dilute acetic acid.

ESBACH'S REAGENT:—

Picric acid	10 grams.
Citric acid	20 grams.
Water	1000 c.c.

Tsuchiya's Reagent:—

Phosphotungstic acid	1.5 grams.
Concentrated HCl	5.0 c.c.
95 per cent. alcohol q. s. ad	100.0 c.c.

Fehling's Reagent:—

The reagent consists of two solutions which are kept in separate bottles until mixed immediately before using.

Solution "A"—Copper sulphate	34.64 grams.
Water	500.00 c.c.
Solution "B"—Sodium-potassium tartrate	173.00 grams.
Sodium hydroxid	125.00 grams.
Water	500.00 c.c.

These solutions are used in equal parts for the test.

Nylander's Reagent:—

Bismuth subnitrate	2 grams.
Rochelle salt	4 grams.
Sodium hydroxid (8 per cent. sol.)	100 c.c.

Trommer-Simrock Reagent:—

Copper sulphate	2 grams.
Potassium hydroxid (5 per cent. sol.)	150 c.c.
Glycerin	15 c.c.
Distilled water	15 c.c.

Purdy's Reagent for Sugar:—

Copper sulphate	4.72 grams.
Glycerin	38.00 c.c.
Water	200.00 c.c.

These should be dissolved in the water by gentle heat.

Potassium hydroxid	23.50 grams.
Water	200.00 c.c.

Dissolve separately and then add to the copper solution. When cold add:—

Ammonia hydroxid (strong)	450.00 c.c.
Water q. s.	1000.00 c.c.

DIAZO REAGENT:—

This reagent consists of two solutions which are kept separate until mixed for the test.

Solution "A"—Sulphanilic acid	1.0	gram.
Hydrochloric acid (con.)	50.0	c.c.
Water	1000.0	c.c.
Solution "B"—Sodium nitrite	1.0	c.c.
Water	200.0	c.c.

Proportion for test: "A," 5 c.c. "B," 3 drops

BENEDICT'S SOLUTION:—

Copper sulphate (C. P. crystallized) ..	17.3	grams.
Sodium or potassium citrate	173.0	grams.
Sodium carbonate (crystallized) or one-half the amount of the anhydrous salt may be used	200.0	grams.
Distilled water .....q. s. ad	1000.00	c.c.

Dissolve the citrate and carbonate (with aid of heat) in about 700 cubic centimeters of water and filter if necessary. Dissolve the $CuSO_4$ in about 100 cubic centimeters of water and pour into the alkaline solution. Cool and make up to 1 liter.

BANG'S SOLUTION.—(*a*) One hundred grams of potassium bicarbonate are dissolved in about 1300 cubic centimeters of distilled water contained in a 2-liter flask. To this solution are added 500 grams of potassium carbonate and 400 grams of potassium sulphocyanate. Exactly 25 grams of pure copper sulphate ($CuSO_4$, $5H_2O$) are then dissolved in about 150 cubic centimeters of warm distilled water. After cooling, this solution is added gradually to the carbonate solution. Add water up to the 2-liter mark, allow to stand for twenty-four hours, and filter. This solution is stable for about three months.

(*b*) Two hundred grams of potassium sulphocyanate are dissolved in about 1500 cubic centimeters in a 2-liter flask; 6.55 grams of hydroxylamin sulphate are dissolved in water and added gradually to the sulphocyanate solution. Add water up to the 2-liter mark and preserve the mixture in dark-colored bottles. This solution is very permanent.

Haine's Solution:—

Copper sulphate	12 grams.
Potassium hydrate	45 grams.
Glycerin	90 c.c.
Water q. s. ad	1000 c.c.

A perfectly clear, transparent, dark-blue liquid results which throws down a very slight reddish deposit of cuprous oxid on standing a week or more. This does not affect the value of the solution, as the clear blue solution is simply decanted as required.

Ruhemann's Iodin Solution:—

Iodin	0.50 grams.
Potassium iodid	1.25 grams.
Absolute alcohol	7.50 c.c.
Glycerin	5.00 c.c.
Distilled water q. s. ad	100.00 c.c.

FOR BLOOD.

Diluting Solution for Counting Blood-Cells. Hayem's Solution for Counting Erythrocytes:—

Mercuric bichlorid	0.5 gram.
Sodium sulphate	5.0 grams.
Sodium chlorid	1.0 gram.
Aq. dest.	200.0 c.c.

Toisson's Solution for Simultaneously Counting Red and White Cells:—

Methyl violet	0.05 gram.
Neutral glycerin	30.00 c.c.
Aq. dest.	80.00 c.c.

Mix and add:—

Sodium chlorid	1.00 gram.
Sodium sulphate	8.00 grams.
Aq. dest.	80.00 c.c.

Filter. Twelve minutes required to stain white blood-cells.

A. E. Osmond[2] recommends the following diluting solutions for the white and red count as being as efficient as the more elaborate diluting solutions, while having the advantage that they do not require a chemist for their preparation. "These solutions may be made offhand."

For counting the red cells, normal salt solution tinged with a drop of eosin.

For the white a 1 per cent. solution of acetic acid tinged with a drop of methylene-blue. He states that a yellow artificial light shows the cells up best, and the only real need for the coloring in these solutions is to distinguish one from the other.

Red Cell Count.—Two and one-half per cent. solution potassium bichromate in distilled water. This preserves the red cells and destroys the white cells. The solution is permanent, but may have to be filtered occasionally to remove sediment.

White Cell Count.—One-half per cent. solution of acetic acid. This solution hemolizes the red cells and accentuates the structure of the white cells. It is stable, but may develop a mold, which may be removed by filtering.

Pitfield's Solution for White Cells.[3]—Acacia gum, 20 grams; distilled water, 500 cubic centimeters; mix, dissolve, and add glacial acetic acid, 50 cubic centimeters, gentian violet, 1 decigram; mix, warm, and filter while warm through a wet filter. This makes a superior diluting fluid for counting leukocytes, because it is viscid and does not flow out of pipettes easily. Thus, these can be filled more accurately because the fluid flows much more slowly. Neat drops of definite size may be slowly put up on the counting chamber. The pipette, filled, may be carried without fear of spilling the contents. The leukocytes do not settle in the mixing chamber quickly and remain evenly distributed throughout the fluid. The end of the pipette must be wet before the fluid can be blown out, as the end of the pipette is quickly sealed with the acacia.

Methods for Fixing Blood and Other Films upon Glass Slides Preparatory to Staining.—1. Equal parts of absolute alcohol and ether. The specimen should be immersed

[2] Lancet-Clinic, November 16, 1912.
[3] New York Medical Journal, July 8, 1910.

in this for half to two hours. This method is particularly good for malarial parasites and degeneration in blood-cells.

2. Absolute alcohol for five minutes.

3. Flemming's solution:—

Chromic acid, 1 per cent.	15 parts.
Osmic acid, 2 per cent.	4 parts.
Glacial acetic acid	1 part.

The blood-specimens, as soon as they are made, before they have time to air-dry, are plunged into this solution and allowed to remain for ten minutes. They are then washed in running water for ten minutes and dried. This solution is very useful for demonstrating the chromatin of nuclei.

4. Vapor of formaldehyde.

5. Heat.

6. Methyl alcohol. This is coming rapidly in favor, as it can be mixed with the stain, thus reducing the time of preparing specimens.

STAINING REAGENTS.

Because of the universal employment of staining methods in the examination of laboratory specimens, these solutions and their preparations have been grouped under the head of Stains on pages 407 to 411.

FOR GASTRIC ANALYSIS.

Uffleman's Reagent:—

Carbolic acid (4 per cent.)	10 c.c.
Water	20 c.c.
Liquor ferri chloridi	1 drop.

This solution should be a clear amethyst color, and should be prepared fresh for use.

Lugol's Iodin Solution:—

Iodin	1 part.
Potassium iodid	2 parts.
Water	50 parts.

Gunzberg's Phloroglucin Vanillin:—

Phloroglucin	2 grams.
Vanillin	1 gram.
Alcohol	30 c.c.

This solution, if active, is pale yellow. It darkens and deteriorates with age, especially on exposure to light, so should be kept in colored bottles and made fresh from time to time.

SPUTUM.

Much's Solution.—Ten cubic centimeters of a saturated alcoholic solution of Gruebler's methyl violet, B. N., mixed with 90 cubic centimeters of a 2 per cent. aqueous carbolic acid solution.

Czaplewsky's Stain.[4]—One gram of fuchsin is dissolved in 5 cubic centimeters of liquid carbolic acid in a dish; 50 cubic centimeters of glycerin are then added, stirring constantly, and finally diluted with water to 100 cubic centimeters. The solution is said to keep extremely well, and does not need to be filtered.

FOR CEREBROSPINAL FLUID AND MILK.

Saturated aqueous solution of methyl violet. (R 5.)

Cream-testing Solutions:—

"A"—Amylic alcohol ..... 37 parts by volume.
Methyl alcohol ..... 13 parts by volume.
Hydrochloric acid ..... 50 parts by volume.

"B"—Sulphuric acid. sp. gr. 1832.

STAINS.

Ehrlich's "Triacid" Stain:—

Orange G.	13.0-14.0 c.c.
Acid fuchsin	6.0- 7.0 c.c.
Distilled water	15.0 c.c.
Methyl green	25.5 c.c.
Alcohol	10.0 c.c.
Glycerin	10.0 c.c.

The three stains, orange G, acid fuchsin, and methyl green, are prepared in saturated aqueous solutions, and then mixed in the above amounts while being shaken thoroughly.

[4] Hyg. Rundschau, No. 21, 1896.

Eosin and Methylene-Blue:—

Eosin ..... 0.5 per cent. in 70 per cent. alcohol.
Stain for a few minutes, wash, blot, and apply.

Methylene-blue ..... 1 per cent. aqueous.
Stain for a few minutes.

Chezinsky Stain:—

Methylene-blue, sat. aq. sol.	45 c.c.
Eosin, 0.5 per cent. in 70 per cent. alcohol ...	25 c.c.
Distilled water	45 c.c.

The specimens are fixed in absolute alcohol for from five to thirty minutes, and stained in a thermostat at 37° C. for from three to six hours.

Eosin and Hematoxylin (Delafield's).—Two solutions are required with equally good results, the eosin solution, mentioned above, and hematoxylin, the formula for which is:—

Hematoxylin crystals	4 grams.
Alcohol (absolute)	25 c.c.
Ammonium-alum crystals C. P.	52 grams.
Distilled water	400 c.c.
Glycerin C. P.	100 c.c.
Methyl alcohol, C. P.	100 c.c.

and which is prepared as follows: Rub the hematoxylin crystals up with the alcohol until they are dissolved; then place the solution in a loosely corked glass bottle, allowing it to stand exposed to the light for four days. Dissolve the ammonium-alum in the water and allow it to stand exposed in the same way for four days. At the end of this time mix the two solutions, shake thoroughly, and filter at the end of three hours. Add the glycerin and methyl alcohol to the filtrate and allow this to stand overnight. Filter the mixture, place it in a clear bottle, and allow it to ripen, exposed to the light for six weeks, when it is ready for use. This stain is applied and allowed to act for from two to four minutes after the eosin stain has been thoroughly washed from the slide.

HEMATOXYLIN-EOSIN (Ehrlich's mixture):—

Eosin (crystals)	0.5 gram.
Hematoxylin	2.0 grams.
Absolute alcohol, Distilled water, Glycerin, of each	100.0 c.c.
Glacial acetic acid	10.0 c.c.

Alum in excess.

This mixture should be allowed to stand for several weeks before it is ready for use. The specimens are stained in from half to two hours.

CARBOL GENTIAN-VIOLET:—

Gentian-violet, conc. alcoholic sol.	10.0 c.c.
Carbolic acid, 5 per cent. watery sol.	100.0 c.c.

GIEMSA IMPROVED STAIN:—

Azur II eosin	3.0 grams.
Azur II	0.8 gram.

Exsiccate, pulverize, and sift.

Dissolve in chemically pure glycerin at 60° C.	250.0 c.c.

When solution is complete add:—

Methyl alcohol at temperature 60° C.	250.0 c.c.

Shake well, allow to stand for twenty-four hours, then filter.

DECOLORIZING SOLUTIONS:—

I. Acetic acid, 0.5 to 5.0 per cent. watery solution.

II. Nitric acid, 20 to 30 per cent.

III. Acid alcohol:—

Sulphuric acid (conc.)	30 drops.
Alcohol (95 per cent.)	50 c.c.
Water	150 c.c.

Löffler's Alkaline Methylene-blue:—

Concent. alcohol solution methylene-blue..	30.0 c.c.
Potassium hydrate ($\frac{1}{10000}$)	100.0 c.c.

Ziehl's Carbol-Fuchsin:—

Fuchsin in substance	1 gram.
Carbolic acid (cryst.)	5 grams.
Alcohol (95 per cent.)	10 c.c.
Distilled water	100 c.c.

Or it may be prepared by adding to a 5 per cent. watery solution of carbolic acid a saturated alcoholic solution of fuchsin until a metallic luster appears on the surface of the liquid.

Gabbott's Method:—

A—Fuchsin	1 gram.
Absolute alcohol	10 c.c.
Carbolic acid (5 per cent.)	100 c.c.
B—Methylene-blue	2 grams.
Sulphuric acid (35 per cent. sol.) ...	100 c.c.

Czaplewsky describes Weigert's modification of Gram's method as follows: Stain for one minute in carbolic gentian-violet (11 cubic centimeters of a concentrated alcoholic solution of gentian-violet, 10 cubic centimeters of alcohol, 50 cubic centimeters of a 5 per cent. solution of carbolic acid, 50 cubic centimeters of distilled water) wash for from thirty to sixty seconds in Lugol's solution, wash, dry, differentiate with anilin-xylol 2:1 to which has been added 1.5 per cent. of acetone; wash with xylol, dry and counter-stain with diluted carbol fuchsin, 1:10 for about one minute, during which time the specimen is warmed slightly. The specimen is then washed, dried, embedded in Canada balsam, and examined with the oil-immersion lens.

GRAM'S IODINE:—

Iodin	1 gm.
Potassium iodid	2 gm.
Distilled water	300 c.c.

KOCH-EHRLICH GENTIAN-VIOLET:—

Take distilled water, 100. cubic centimeters, and add anilin oil, drop by drop, until the solution has an opalescent appearance. The vessel containing the mixture should be thoroughly shaken after the addition of each drop. It is then filtered through moistened filter paper until the filtrate is clear. To 100 cubic centimeters of the filtrate add 10 cubic centimeters of absolute alcohol and 2 cubic centimeters of concentrated solution of gentian-violet.

UNNA'S ORCEIN STAIN:—

Orcein in substance	1 gm.
Hydrochloric acid	1 c.c.
Absolute alcohol	100 c.c.

REAGENTS FOR STAINING FLAGELLA:—

Mordant.

Tannic acid (20 ac. to 80 water)	10 c.c.
Ferric sulphate cold sat. sol.	5 c.c.
Fuchsin sat. watery sol.	1 c.c.

Adjuvants.

Sodium hydrate 1 per cent. aqueous solution.

Sulphuric acid (1 c.c. equal 1 c.c. of 1 per cent. NaOH).

PAPPENHEIM'S SOLUTION:—

Corallin	1 part.
Absolute alcohol	100 parts.

Add to the above solution methylene-blue in bulk to saturation. Finally add 20 parts of glycerin.

LOEFFLER'S MORDANT:—

Tannic acid solution (2 parts acid, 80 parts water)	10. c.c.
Ferrous sulphate saturated solution	5. c.c.
Fuchsin saturated aqueous solution	1. c.c.

Ebner's Fluid:—

Hydrochloric acid	2.5 c.cm.
Sodium chlorid, C. P.	2.5 c.cm.
Distilled water	100.0 c.cm.
Alcohol, 95 per cent.	500.0 c.cm.

Labeling Smears:—

As it often occurs that a number of slides are made at one time, or a number of slides from one patient are taken at different hours, it is necessary that such slides should be labeled at once. The most convenient method is that of writing on the end or back of a slide with ordinary ink. This should be quite dry before the staining process is begun, then there will be no fear of it washing off. Another method suggested by Powell is: After making a dry film, the name, date, and other necessary information are scratched on the film, with the head or point of a needle, the film used being so extensive that the writing in no way interferes with subsequent study. In place of the needle, R. H. von Ezdorf[5] suggests the use of an ordinary black lead pencil, preferably soft. The label thus made on the blood film being a carbon deposit remains permanent and is not affected by the staining and washing of the slide.

A New and Stable Solution of Gentian-Violet:—

The decided tendency of the average gentian-violet solution to decompose, especially in warm weather, is a difficulty frequently encountered by the laboratory worker. The result is loss of the entire solution and much time required to make a new one. The following suggestions are practical and obviate this difficulty.

Dr. E. Burvill-Holmes has had success with the addition of 3 to 5 per cent. of glycerin to the stain which improves its stability if kept in a dark, cool place. Muir and Ritchie recommend the use of phenol water 1 part in 10. These methods while being improvements do not prevent decomposition of the stain. Robert Kilduffe recommends the following preparation which he has found to work admirably. Two stock solutions are employed: A. 5 cubic centimeters 40 per cent. formaldehyde are added to 95 cubic centimeters of distilled water. B.

[5] Jour. A. M. A., Jan. 8, 1910.

Saturated alcoholic solution of gentian-violet. Mixing these in the proportion of 25 parts of B and 75 parts of A such a solution has been kept for a long time at ordinary temperatures without deterioration. The advantages of this solution are said to be: (1) It does not decompose. (2) Moulds cannot grow in it. (3) No modification of technic necessary. (4) Preparations made with it are sterile.

Universal Staining Method:—

The discovery of the process whereby two or more colors could be chemically combined in one staining reagent, marked a great advance in the field of hematology. It affords greater opportunity for more detailed study of the minute structure of the cells of the blood, which in turn has resulted in a better classification of the different elements through the differentiation of new and distinct varieties which, until recently, have been unrecognized. Of these combination stains the Polychrome or "universal" staining method is by far the best and most practical, and is now rapidly superseding the older and more cumbersome methods. For this reason it becomes a matter of considerable importance, almost a necessity, that the clinical worker should have at hand one or more of these stains ready for immediate use when occasion requires.

Unfortunately the preparation of this class of stain involves considerable expenditure of time, and demands no small amount of chemical knowledge and manipulative skill. These factors combine to limit the preparation of these stains to a comparatively small number of experienced workers, while the majority of the profession is dependent upon unstable liquid stains, obtainable through the supply houses, the composition of which is frequently so variable as to render the results valueless. Further, all aniline stains of this character are prone to decomposition when kept even for a moderately long time in solution.

Fortunately the demand for a uniform and reliable stain has recently been met by a London Pharmaceutical House,[6] who now carry in stock a number of very uniform and perfectly reliable stains under the name of "Soloid" brand. These stains are very carefully prepared and their composition practically

[6] Burroughs Wellcome & Co., with a branch at 45 Lafayette Street, New York City.

uniform. A definite quantity of the dried stain is compressed into a tiny tablet and dispensed in vials containing six. Each package is accompanied with information indicating the proper dilution and best working conditions for that particular stain.

The "Soloid" stains permit of the preparation of small quantities of liquid stain whereby waste through evaporation or decomposition is reduced to a minimum; at the same time the results obtained, as far as the author has employed them, are in every way satisfactory.

Practically all the stains in common use, as well as the rare ones, are now prepared by this manufacturer, together with appropriate mordants, decolorizers, etc. These may be obtained of any reliable drug house or instrument dealer.

The Polychrome Methylene-Blue-Eosin Stains (Romanowski).—There are about fifteen different modifications of this stain. The majority of them are difficult of manufacture, even by an expert, so it is recommended that they be bought ready-made from the laboratory apparatus and supply houses which make them.

For those who desire to make this stain for themselves, the Wright stain and a modification by Hastings,[7] are appended, the two having the greatest popularity.

All the Romanowski stains are made with wood alcohol, which, during the first portion of its application, acts as a fixative.

Hastings' Stain.—The dry stains necessary are eosin (water solution), yellow (Gruebler), and methylene-blue (Ehrlich's rectif.) (Gruebler).

Solution "A"—Eosin 1 per cent. aqueous.
Solution "B"—Alkaline methylene-blue 1 per cent. aqueous.
Solution "C"—Methylene-blue 1 per cent. aqueous.

Solution "A" may be kept ready-made, solutions "B" and "C" must be made fresh.

To prepare solution "B," use a warm 1 per cent. solution of dry powdered sodium carbonate. Add to it one per cent. of methylene-blue powder, and heat over a water bath for 15

[7] Hastings: Johns Hopkins Hospital Bulletin, 1905.

minutes. Add 30 cubic centimeters of water for each 100 cubic centimeters of the original fluid, and heat again for 15 minutes. Then pour off the solution from the residue and divide into two equal parts. To one part add enough 12.5 per cent. acetic acid solution to make a faintly acid reaction. This is best determined by taking a piece of blue litmus paper and allowing a drop to fall upon it, taking as the end reaction the point at which the margin of the drop after absorption in the paper shows a faint pink. Then add the remaining unneutralized portion to this.

To mix the stain use distilled water, 1000 cubic centimeters; Solution "A," 100 cubic centimeters; solution "B," 200 cubic centimeters; solution "C," 70 to 80 cubic centimeters. In adding solution "C" put in 70 cubic centimeters at once, and stir well; if no precipitate appears, add 1 cubic centimeter at a time until one does appear. After the precipitate appears the stain is allowed to stand for half an hour and then filtered through one filter. Forced filtration is generally necessary.

The dry residue is removed from the paper and pulverized. It may be kept in this form or dissolved in Merck's pure methyl alcohol. Seven- to nine- tenths of a gram of dried stain is usually obtained. Three-tenths of a grain is dissolved in 100 cubic centimeters of alcohol for the staining solution. In dissolving the stain it must be rubbed up with the alcohol in a mortar, as the powder is with difficulty soluble.

If there are more than nine-tenths gram of the dried stain obtained, the preparation is useless, and its preparation should be begun again.

For each new lot of stain made up, one must determine the relative proportion of stain and water used in staining, and the relative lengths of time during which the pure and diluted stain is allowed to act.

Usually 2 drops of stain on the smear for one minute and then 4 drops of water added and allowed to act for four minutes gives the best result.

For uniformity in dropping a dropper should be used.

Wright's Stain.—To a 0.5 per cent. aqueous solution of sodium bicarbonate add methylene-blue (B. X. or "medici-

nally pure") in the proportion of 1 gram of the dye to each 100 cubic centimeters of the solution. Heat the mixture in a steam sterilizer at 100° C. for one full hour, counting the time after the sterilizer has become thoroughly heated. The mixture is to be contained in a flask or flasks of such a size and shape that it forms a layer not more than 6 centimeters deep. After heating, allow the mixture to cool, placing the flask in cold water if desired, and then filter it to remove the precipitate which has formed in it. It should, when cold, have a deep purple-red color when viewed in a thin layer by transmitted yellowish artificial light. It does not show this color while it is warm. To each 100 cubic centimeters of the filtered mixture add 500 cubic centimeters of a 0.1 per cent. aqueous solution of "yellowish water soluble" eosin and mix thoroughly. Collect the abundant precipitate, which immediately appears, on a filter. When the precipitate is dry, dissolve it in methyl alcohol (Merck's reagent) in the proportion of 0.1 gram to 60 cubic centimeters of the alcohol. In order to facilitate solution, the precipitate is to be rubbed up with the alcohol in a porcelain dish or mortar with a spatula or pestle. This alcoholic solution of the precipitate is the staining fluid. It should be kept in a well-stoppered bottle because of the volatility of the alcohol.[8]

All polychrome methylene-blue stains require experiment, since different mixtures by the same method require slight variations in their use. These must be ascertained by trial. Use distilled water to wash the specimen, since tap-water may ruin it.

NORMAL SOLUTIONS, STANDARD SOLUTIONS, AND DECINORMAL SOLUTIONS.

A *normal solution* ($\frac{n}{1}$) in substance is one which contains the hydrogen equivalent of its molecular weight dissolved in a liter of water, hydrogen being considered for analytical purposes as 1.

Equal volumes of normal solutions should combine exactly with each other; for example, 1 cubic centimeter of $\frac{n}{1}$ hydrochloric acid should exactly neutralize 1 cubic centimeter of $\frac{n}{1}$

[8] Jour. A. M. A., vol. LV, 1910, p. 1979.

sodium or potassium hydrate or 10 cubic centimeters of $\frac{n}{10}$ alkali.

A *decinormal* ($\frac{n}{10}$) solution is a solution of such a strength that 10 cubic centimeters of alkali will exactly neutralize 1 cubic centimeter of a $\frac{n}{1}$ solution of acid. Decinormal solutions are usually prepared by diluting a $\frac{n}{1}$ solution with 9 parts of distilled water; then if absolute accuracy is demanded it should be titrated and standardized with a normal solution of authentic accuracy.

A *standard solution* is one having an arbitrary amount of a chemical substance in solution, this amount being usually determined by convention, or arranged for convenience in performing certain tests.

These solutions may be obtained accurately standardized from reliable manufacturers.

Preparation of Decinormal Solutions.—*A normal sodium hydrate solution* is one which contains in each liter as many grams of the substance in question as its equivalent weight. A decinormal $\frac{n}{10}$ solution is made from this by diluting 10 times; because of the facility with which this substance takes up water, and so is constantly changing in weight, it is best to start with a normal oxalic acid solution as a basis.

The molecular weight of oxalic acid is 126.024 and, as the acid is dibasic, a normal solution would contain one-half of the molecular weight; that is, 63.012 grams. For ordinary work the commercial acid may be assumed as sufficiently pure to use as standard. Sixty-three grams of a well-crystallized and chemically pure oxalic acid are dissolved in distilled water and the volume made up to exactly 1 liter. Now, a normal sodium hydroxid solution will require, in order to neutralize a given volume of the normal oxalic acid solution, exactly the same volume. A few drops of alcoholic phenolphthalein solution are added as an indicator to 10 cubic centimeters of normal oxalic acid solution. Then to this an approximately normal sodium hydroxid solution (40 grams dissolved in 900 cubic centimeters water) is added from a buret until the acid is neutralized—*i.e.*, until the mixture takes on a permanent reddish color. If the normal NaOH solution is correct, it will require exactly 10 cubic centimeters.

Generally speaking, less is needed. For instance, if we employed 9.5 cubic centimeters of the $\frac{n}{10}$ NaOH for neutralization, we must add 0.5 cubic centimeter of water to each 9.5 cubic centimeters of the solution. Then 10 cubic centimeters of the normal NaOH solution will correspond to exactly 10 cubic centimeters of the normal oxalic acid solution. From this we can easily estimate how much water to add to the 1000 cubic centimeters of solution—*i.e.*, $\frac{1000}{9.5} \times 0.5$. Normal sodium hydroxid solution is too strong to use for the titration of the gastric juice; hence we prepare $\frac{n}{10}$ by diluting the normal solution with 9 parts distilled water.

Decinormal Hydrochloric Acid Solution.—This solution is prepared by diluting 15 cubic centimeters of C. P. hydrochloric acid up to 1000 cubic centimeters with distilled water; 10 cubic centimeters of this is then titrated with the standardized $\frac{n}{10}$ solution of sodium hydrate and corrected according to the method outlined above.

Potassium sulphocyanide and standard *silver nitrate solution.* A solution of potassium sulphocyanid is used in the determination of the chlorids in the urine and other fluids by the Volhard method. A special solution containing 16.6 grams of the sulphocyanid to the liter, which corresponds to a special silver nitrate solution 1 cubic centimeter of which is equivalent to 1 centigram of sodium chlorid, is prepared.

As the potassium sulphocyanid is hygroscopic, a standard solution cannot be made up by direct weighing, but only by titration against a previously prepared standard silver solution. The reaction between silver nitrate and potassium sulphocyanid is $AgNO_3 + KSCN = AgSCN + KNO_3$. In other words, one molecule of silver nitrate weighing 169.97 is precipitated by a molecule of potassium sulphocyanid weighing 97.22. As the silver nitrate solution corresponding to 1 centigram of sodium chlorid contains 29.06 grams to the liter, the sulphocyanid solution which corresponds to this should be 16.6 grams to the liter.

For the special silver nitrate solution corresponding to 1 centigram of sodium chlorid contains 169.97, divided by 5.85, which equals 29.06 grams to the liter, so the corresponding

potassium sulphocyanid solution would contain 97.22, divided by 5.85, which equals 16.6 grams to the liter.

About 18 grams of the sulphocyanid are weighed out and dissolved in about 900 cubic centimeters of water. Ten cubic centimeters of the standard silver solution are diluted to 100 with water. Four cubic centimeters of nitric acid and 5 cubic centimeters of ammonioferric alum solution are added and the mixture is titrated with the potassium sulphocyanid of unknown strength. The end-reaction is marked by the production of a slight red color, which remains on stirring the fluid. Inasmuch as the sulphocyanid solution has been purposely made too strong, less than 10 cubic centimeters will be required to neutralize 10 cubic centimeters of the silver solution. If we assume that 9.8 cubic centimeters of the sulphocyanid are used, then it is evident that to each 9.8 cubic centimeters of the solution 10 minus 9.8 cubic centimeters, or 0.2 cubic centimeter of water, should be added to bring the solution to the proper strength. Nine hundred and eighty cubic centimeters of the fluid are measured off and filled up to a thousand with distilled water. Or, 1000 cubic centimeters are measured off and 24.08 cubic centimeters of distilled water are added from a burette. The contents of the flask is thoroughly shaken; a fresh sample is withdrawn and titrated against another 10 cubic centimeters of silver nitrate. The second dilution will probably require only the addition of a few cubic centimeters of water, in order to bring it to the standard. If preferred, however, the solution may be left of an arbitrary strength and the correction made at the time of titrating the urine.

SPECIAL METHODS AND REACTIONS.

Modified Guaiac Test.—L. de Jager,[9] in order to bring out more plainly the blue color denoting a positive result in the guaiac test for blood in the feces proceeds as follows: A piece of the feces the size of a pea is inserted in a large test-tube with 30 per cent. acetic acid and the tube filled about two-thirds with the acid. Ether is then added, the tube shaken up and allowed to stand until the ether rises to the surfaces; the addition of a

[9] Zentralblatt für innere Med., June 22, 1912.

few drops of alcohol, together with gentle stirring, will hasten the separation of the ether. To 5 cubic centimeters of the ether solution are then added 5 drops of a 20 per cent. sodium hydroxid solution, 10 drops of freshly prepared guaiac solution, and 2 cubic centimeters of a 3 per cent. solution of hydrogen peroxid. Where a small amount of blood is present there develops a distinct green color; if the amount is large, indigo blue. Thus performed, the guaiac test was found to be more sensitive than where the hydroxid had not been added, 4 parts of blood in 100,000 giving a distinctly positive and prompt result, as against 6 to 100,000 where the alkali is omitted.

Phenolphthalin Test for Blood.—This test, originally employed by Meyer and later by Utz, has been so improved by Kastle, Amoss, and Benoit that it is now probably the most delicate one for the detection of blood, especially in stains, now available, its limit of delicacy being about 1 part of blood in 8 million of water. As in the other tests, the hemoglobin acts as an oxygen carrier, the active oxidizing agents being ozonized turpentine or hydrogen peroxid.

Phenolphthalin is a product of the reduction of phenolphthalein by zinc in alkaline solution. When oxidized in alkaline solution, it is converted into phenolphthalein with production of an intense red color. The reagent may be obtained in the market or, preferably, prepared as follows: Phenolphthalein is dissolved to considerable excess of 30 per cent. sodium hydrate solution and boiled with an excess of zinc dust until a few drops of the strongly alkaline liquid no longer gives a red color after neutralization with HCl and sufficient alkali to alkalinize the solution. The solution is then decanted from the excess of zinc dust and the phenolphthalin is precipitated by acidifying with HCl. Collect the precipitate on a filter and purify by repeated crystallization from water and alcohol. This purification is continued until a white, crystalline compound is obtained free from every trace of phenolphthalein (as shown by absence of red coloration on addition of alkali). Dry at room temperature or in the oven at 50° to 80° C., care being taken to avoid contact with metallic surfaces. This compound should be kept in tightly stoppered bottles in a dark place, as oxidation gradually occurs.

The solution, as used in the test, is as follows: Mix a slight excess of phenolphthalin, prepared as above, with 1 cubic centimeter of $\frac{n}{10}$ sodium hydrate solution and a few cubic centimeters of redistilled (from glass) water, shake thoroughly and filter. To the filtrate add 20 cubic centimeters of $\frac{n}{10}$ NaOH solution, 0.1 cubic centimeter of 3 per cent. hydrogen peroxid solution, and make the mixture up to 100 cubic centimeters. This solution should show no trace of pink coloration when fresh, but gradually acquires a color, which may become so intense that the reagent cannot be employed. In forensic work use only freshly prepared solutions.

To 1 part of the aqueous solution of the stain or of the secretion or excretion to be tested add 2 parts of the reagent and allow to stand for a few minutes. In the presence of blood a pink-red color appears, the intensity depending on the amount of blood present. This reaction is retarded by the extracts of various animal tissues or various secretions of the body. For this reason we are never able to detect as easily small amounts of blood in the secretions as in watery solutions of pure blood, the limit of delicacy of all reactions being far less in the former than in the latter case. Boiling the solutions before applying the test removed most of the interfering factors. If the secretion be treated with a thick cream of aluminum-hydrate suspension, the precipitate will carry down the blood-pigment and thus concentrate it. A small amount of this precipitate, whether derived from saliva, urine, feces suspension, milk, or exudates, will show a decided red color when added to 2 cubic centimeters of the reagent. Of course, in applying the test to the aqueous solution of the blood stain, no such treatment need be employed.

METHOD OF STAINING CAPSULATED BACTERIA IN BODY FLUIDS.

Smith[10] describes his method of staining capsulated bacteria in body fluids as follows: Make a thin smear from fresh sputum, lung, pleural, or pericardial exudate. Pass through flame. Cover with 10 per cent. aqueous solution of phosphomolybdic acid four to five seconds. Wash in water. If the

[10] Boston Med. and Surg. Jour., Nov. 24, 1910.

micro-organism is Gram staining, like the pneumococcus or *Streptococcus mucosus capsulatus,* stain with anilin oil-gentian violet, steaming for from one-quarter to one-half minute. Wash in water. Treat with Gram's solution of iodin; steam for from one-quarter to one-half minute. Decolorize with 95 per cent. alcohol. Wash in water. Stain with 6 per cent. aqueous solution of eosin, for from one-half to one minute, warming gently. Wash in water. Wash in absolute alcohol. Clear in xylol and mount in Canada balsam. The capsule will be found to be distinct, clear cut, eosin stained, about the Gram-stained micro-organism. If the micro-organism is Gram decolorized; after covering the smear with phosphomolybdic acid and washing, stain with 6 per cent. aqueous solution of eosin, for from one-half to one minute, warming gently. Wash in water. Counter-stain with Löffler's methylene-blue, for from one-quarter to one-half minute, warming gently. Wash in absolute alcohol. Clear in xylol and mount in Canada balsam. The capsule will appear eosin stained about the blue-stained micro-organism.

Welch's Method.[11]—1. Smear the sputum without the addition of any water, dry, and fix by a moderate amount of heat.

2. Flood the smear with glacial acetic acid and immediately pour off.

3. Wash off the acid with anilin water-gentian violet.

4. Replace the stain with 2 per cent. salt solution, or examine directly in the stain by covering and pressing out the excess of dry, so that enough light will pass through to permit of examination. Do not wash with water. The capsule can be demonstrated only in fresh sputum, and not constantly in that.

Hiss's Method.[12]—1. Smear the sputum in a very thin layer without the addition of water, dry and fix by heat.

2. Cover the preparation with a mixture containing 5 cubic centimeters of a saturated alcoholic solution of gentian violet in 95 cubic centimeters of distilled water. Heat till steam begins to rise.

3. Wash off the dye with a 20 per cent. solution of copper sulphate.

4. Dry and mount in dammar.

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[11] Bull. Johns Hopkins Hosp., 1892, p. 125.
[12] Wood's "Chemical and Microscopical Diagnosis," 2d ed., 1910.

Multiples of a Grain

From 1 grain to 1 ounce

U. S. A.	Metric	U. S. A.	Metric
gr. 1	0.065 gm.	gr. 15	0.972 gm.
gr. 1⅙	0.086 gm.	gr. 18	1.166 gm.
gr. 1½	0.097 gm.	gr. 20	1.296 gm.
gr. 1¾	0.113 gm.	gr. 25	1.620 gm.
gr. 2	0.13 gm.	gr. 30	1.944 gm.
gr. 2½	0.162 gm.	gr. 35	2.268 gm.
gr. 3	0.194 gm.	gr. 40	2.592 gm.
gr. 3½	0.227 gm.	gr. 50	3.24 gm.
gr. 4	0.259 gm.	gr. 60	3.89 gm.
gr. 5	0.324 gm.	gr. 120	7.78 gm.
gr. 6	0.389 gm.	oz. ⅛	3.54 gm.
gr. 7	0.454 gm.	oz. ¼	7.08 gm.
gr. 8	0.518 gm.	oz. ½	14.17 gm.
gr. 8¾	0.567 gm.	dr. 4	15.55 gm.
gr. 9	0.583 gm.	oz. 1	28.35 gm.
gr. 10	0.648 gm.	dr. 8	31.1 gm.
gr. 12	0.778 gm.		

Equivalents of U. S. A. and Metric Measures of Capacity

From half-a-minim to 1 fluid ounce

U. S. A.	Metric	U.S.A.	Metric
min. ½	0.03 c.c.	min. 20	1.232 c.c.
min. 1	0.062 c.c.	min. 25	1.54 c.c.
min. 2	0.123 c.c.	min. 30	1.848 c.c.
min. 3	0.185 c.c.	min. 35	2.156 c.c.
min. 4	0.246 c.c.	min. 40	2.464 c.c.
min. 5	0.308 c.c.	min. 50	3.08 c.c.
min. 6	0.370 c.c.	min. 60	3.70 c.c.
min. 7	0.431 c.c.	min. 90	5.54 c.c.
min. 8	0.493 c.c.	min. 120	7.39 c.c.
min. 9	0.554 c.c.	min. 180	11.09 c.c.
min. 10	0.616 c.c.	min. 240	14.79 c.c.
min. 12	0.739 c.c.	min. 360	22.18 c.c.
min. 15	0 924 c.c.	min. 480	29.57 c.c.

In Continental prescribing, a smaller quantity than half a cubic centimeter is usually expressed in drops, which, in dispensing, are dropped from pipette into the cubic centimeter measure.

Approximate U. S. A. Equivalents of Metric Measure of Capacity

Metric	U. S. A.	Metric	U. S. A.
1 c.c.	16 (16.23) min.	25 c.c.	6 fl. dr., 46 min.
2 c.c.	32½ min.	30 c.c.	8 fl. dr., 7 min.
3 c.c.	48¾ min.	40 c.c.	1 fl. oz., 2 fl. dr., 49 min.
4 c.c.	1 fl. dr. 5 min.	50 c.c.	1 fl. oz., 5 fl. dr., 32 min.
5 c.c.	1 fl. dr. 21 min.	75 c.c.	2 fl. oz., 4 fl. dr., 17 min.
6 c.c.	1 fl. dr. 37 min.	100 c.c.	3 fl. oz., 3 fl. dr., 3 min.
7 c.c.	1 fl. dr. 54 min.	125 c.c.	4 fl. oz., 1 fl. dr., 49 min.
8 c.c.	2 fl. dr. 10 min.	150 c.c.	5 fl. oz., 0 fl. dr., 35 min.
9 c.c.	2 fl. dr. 26 min.	200 c.c.	6 fl. oz., 6 fl. dr., 6 min.
10 c.c.	2 fl. dr. 42 min.	300 c.c.	10 fl. oz., 1 fl. dr., 9 min.
12 c.c.	3 fl. dr. 23 min.	500 c.c.	16 fl. oz., 7 fl. dr., 15 min.
15 c.c.	4 fl. dr. 4 min.	1 litre	33 fl. oz., 6 fl. dr., 31 min.
20 c.c.	5 fl. dr. 25 min.		

Approximate U. S. A. Equivalents of Metric Measures of Mass

Metric	U. S. A.	Metric	U. S. A.
1 mgm	1/64 gr.	1 gm.	15½ (15.432) gr.
2 mgm	1/32 gr.	2 gm	30⅞ gr.
3 mgm	1/21 gr	3 gm	46¼ gr.
4 mgm	1/16 gr	4 gm	61¾ gr.
5 mgm	1/13 gr.	5 gm	77⅛ gr.
6.5 mgm	1/10 gr.	7.5 gm	115¾ gr.
8 mgm	⅛ gr.	10 gm	154⅓ gr.
1 cgm	1/6 gr.	15 gm	231½ gr.
2 cgm	⅓ gr	20 gm	308⅔ gr.
3 cgm	½ gr.	25 gm	385⅘ gr.
5 cgm	¾ gr.	30 gm	1 oz. 25½ gr.
6.5 cgm	1 gr.	40 gm	1 oz. 179⅕ gr.
10 cgm	1½ gr.	50 gm	1 oz. 334 gr.
15 cgm	2¼ gr.	75 gm	2 oz. 282½ gr.
20 cgm	3 gr.	100 gm	3 oz. 230¾ gr.
26 cgm	4 gr.	150 gm	5 oz. 127½ gr.
30 cgm	4½ gr.	250 gm	8 oz. 358 gr.
40 cgm	6⅛ gr.	500 gm	1 lb. 1 oz. 278 gr.
50 cgm	7¾ gr.	750 gm	1 lb. 10 oz. 200 gr.
75 cgm	11½ gr.	1 kgm	2 lb. 3 oz. 120 gr.

TABLE FOR CONVERTING APOTHECARIES' WEIGHTS AND MEASURES INTO GRAMS.[13]

Troy Weight.	Metric.	Apothecaries' Measure.	Grams for Liquids.		
Grains.	Grams.		Lighter than Water.	Specific Gravity of Water.	Heavier than Water.
1-400	.00016	♏ 1	.055	.06	.08
1-200	.00033	2	.10	.12	.15
1-128	.0005	3	.16	.18	.24
1-100	.00065	4	.22	.24	.32
1-64	.001	5	.28	.30	.40
1-40	.0015	6	.32	.36	.48
1-30	.002	7	.38	.42	.55
1-20	.003	8	.45	.50	.65
1-16	.004	9	.50	.55	.73
1-12	.005	10	.55	.60	.80
1-10	.006	15	.80	.72	.96
1-8	.008	16	.90	1.00	1.32
1-3	.010	20	1.12	1.25	1.60
1-4	.016	25	1.40	1.55	2.00
1-4	.02	30	1.70	1.90	2.50
1-4	.03	35	2.00	2.20	2.90
1	.065	40	2.25	2.50	3.30
2	.13	48	2.70	3.00	4 00
3	.20	50	2.80	3.12	4.15
4	.26	60 f℥j	3.40	3.75	5.00
5	.32	72	4.00	4.50	6.00
6	.39	80	4.50	5.00	6.65
8	.52	90	5.10	5.60	7.50
10	.65	96	5.40	6.00	8.00
15	1.00	100	5.60	6.25	8.30
20 ℥j	1.30	120 f℥ij	6 75	7.50	10.00
24	1.50	160	9.00	10.00	13.30
26	1.62	180 f℥iij	10.10	11.25	15.00
30 ℥ss	1 95	240 f℥ss	13.50	15.00	20.00
40	2 60	f℥v	16.90	18.75	25.00
50	3.20	f℥vj	20.25	22.50	30.00
60 ℥j	3.90	f℥vij	23.60	26.25	35.00
120 ℥ij	7 80	f℥j	27.00	30.00	40.00
180	11 65	f℥ij	54.00	60.00	80.00
240 ℥ss	15.50	f℥iij	81.00	90.00	120.00
300	19.40	f℥iv	108.00	120.00	160.00
360	23.30	f℥v	135.00	150.00	200.00
420	27.20	f℥vj	162.00	180.00	240 00
480 ℥j	31.10	f℥viij	216.00	240.00	320.00

METRIC WEIGHTS AND MEASURES.

Weights.

1 millgram	0.001 grams	0.015 grains Troy.
1 centigram	0 01 "	0.154 " "
1 decigram	0.1 "	1.543 " "
1 gram		15.432 " "
1 decagram	10 "	154.324 " "
1 hectogram	100 "	0.268 pounds "
1 kilogram	1000 "	2.679 " "

Measures.

1 millimeter	0.001 meter	0.0394 inch.
1 centimeter	0.01 "	0,3937 "
1 decimeter	0.1 "	3.9371 inches.
1 meter		39.3708 "
1 decameter	10 "	32.8089 feet.
1 hectometer	100 "	328.089 "
1 kilometer	1000 "	0.6214 mile.
1 yard or 36 inches		0.9144 meter.
1 inch		25.4 millimeters.

13 From Gould's Pocket Dictionary.

COMPARISON OF THERMOMETERS.[14]

FAHR.	CENT.	REAU.	FAHR.	CENT.	REAU.
212	100	80	76	24.4	19.6
210	98.9	79 1	74	23.3	18.7
208	97 8	78.2	72	22.2	17.8
206	96 7	77.3	70	21.1	16 9
204	95.6	76.4	68	20	15
202	94.4	75.6	66	18.9	15.1
200	93 3	74.7	64	17.8	14.2
198	92.2	73.8	62	16.7	13.3
196	91.1	72.9	60	15 6	12.4
194	90	72	58	14 4	11.6
192	88.9	71.1	56	13.3	10.7
190	87.8	70.2	54	12.2	9.8
188	86 7	69.3	52	11.1	8.9
186	85.6	68 4	50	10	8
184	84.4	67.6	48	8.9	7.1
182	83.3	66.7	46	7.8	6.2
180	8 2.2	65.8	44	6.7	5.3
178	81.1	64.9	42	5 6	4.4
176	80	64	40	4.4	3 6
174	78 9	63.1	38	3 3	2.7
172	77.8	62.2	36	2 2	1.8
170	76.7	61.3	34	1.1	0.9
168	75 6	60.4	32	0	0
166	74.4	59.6	30	-1.1	-0.0
164	73.3	58 7	28	-2.2	-1.8
162	72.2	57.8	26	-3.3	-2.7
160	71.1	56 9	24	-4.4	-3.6
158	70	56	22	-5.6	-4 4
156	68 9	55.1	20	-6.7	-5.3
154	67.8	54.2	18	-7.8	-6.2
152	66 7	53 3	16	-8.9	-7.1
150	65.6	52.4	14	-10	-8
148	64.4	51 6	12	-11 1	8.9
146	63.3	50.7	10	-12.2	-9.8
144	62.2	49.8	8	-13.3	-10.7
142	61.1	48.9	6	-14.4	-11.6
140	60	48	4	-15 6	-12.4
138	58.9	47.1	2	-16.7	-13.3
136	57 8	46 2	0	-17.8	-14.2
134	56.7	45.3	-2	-18.9	-15.1
1 2	55 6	44 4	-4	-20	-16
130	54.4	43.6	-6	-21.1	-16.9
128	53.3	42 7	-8	-22.2	-17.8
126	52.2	41.8	-10	-23.3	-18.7
124	51.1	40.9	-12	-24 4	-19.6
122	50	40	-14	-25.6	-20.4
120	48.9	39.1	-16	-26.7	-21.3
118	47 8	38.2	-18	-27.8	-22.2
116	46.7	37.3	-20	-28.9	-23.1
114	45.6	36 4	-22	-30	-24
112	44.4	35.6	-24	-31.1	-24.9
110	4 .3	34.7	-26	-32 2	-25.8
108	42 2	33.8	-28	-33 3	-26.7
106	41.1	32 9	-30	-34.4	-27.6
104	40	32	-32	-35.6	-28.4
102	38.9	31.1	-34	-36.7	-29.3
100	37 8	30 2	-36	-37.8	-30.2
98	36.7	29.3	-38	-38.9	-31.1
96	35.6	28 4	-40	-40	-32
94	34 4	27 6	-42	-41.1	-32.9
92	33.3	26.7	-44	-42 2	-33.8
90	32 2	25 8	-46	-43.3	-34.7
88	31.1	24.9	-48	-44.4	-35.6
86	30	24	-50	-45.6	-36.4
84	28 9	23.1	-52	-46.7	-37.3
82	2 .8	22 2	-54	-47 8	-38.2
80	26.7	21 3	-56	-48 9	-39.1
78	25.6	20.4			

[14] From Gould's New Medical Dictionary.

DISEASES IN WHICH LABORATORY TESTS ARE OF ESPECIAL VALUE.[15]

The diseases are given in alphabetical order. The principal objects to be examined are enumerated; words in light-face capitals represent the most important laboratory findings. Words in black face represent tests which will usually require the services of a laboratory specialist.

ACTINOMYCOSIS—
1. Pus from abscess (or sputum)—SULPHUR GRANULES OF THE RAY FUNGUS.

AMEBIC DYSENTERY amebiasis.
1. Feces and pus from abscesses—AMŒBA DYSENTERIÆ OR ENTAMŒBA HISTOLYTICA.

ANEMIA—
1. Blood—ABSOLUTE AND DIFFERENTIAL LEUKOCYTE COUNT.
—HEMOGLOBIN TEST.
—BLOOD-PICTURE—microscopic.

ANIMAL PARASITES—
1. Blood—BLOOD-PICTURE—esp. eosinophilia and parasites.
—HEMOGLOBIN.
2. Feces—PARASITES OR OVA.
—OCCULT BLOOD.

ANTHRAX—
1. Purulent discharge—BACTERIA.
2. Blood-culture—BACTERIA.

APPENDICITIS—
1. Blood—ABSOLUTE AND DIFFERENTIAL LEUKOCYTE COUNT.

ASTHMA (bronchial).
1. Blood-picture—EOSINOPHILIA.
2. Sputum—EOSINOPHILIC LEUKOCYTES.
—CURSCHMAN'S SPIRALS.
—CHARCOT-LEYDEN CRYSTALS.

BRAIN TUMORS
or
TUMOR-LIKE PROCESSES.
1. Blood-picture—LYMPHOCYTOSIS.

[15] Taken from "Model Laboratory" by Henry Alberts M.D., and Mildred E. Schuty M.D., Jour. Iowa State Med. Soc., May, 1914.

BRAIN TUMORS or TUMOR-LIKE PROCESSES (*continued*).
2. Cerebro-spinal fluid—NOGUCHI'S BUTYRIC ACID TEST (**Positive Wassermann**) } in syphilis.
—TRACES OF BLOOD—in cerebral hemorrhage.
3. Urine—TRANSITORY GLYCOSURIA.

BRONCHIECTASIS.
1. Sputum—TRISEDIMENTATION.
—PUS CELLS.
—FATTY ACID CRYSTALS.

BRONCHITIS. **Capillary.**
1. Sputum—MUCOID OR MUCOPURULENT.
—PUS CELLS AND EOSINOPHILES.
—BACTERIA.

BRONCHO-PNEUMONIA.
Same as capillary bronchitis.

BUBONIC PLAGUE.
1. Blood—**Slow coagulation time.**
—ABSOLUTE AND DIFFERENTIAL LEUKOCYTE COUNT.
—BACTERIA.

CANCER.
1. **In general—Tissue examination.**
2. Of stomach.
A—Gastric contents—ABSENCE OF FREE HYDROCHLORIC ACID.
—INCREASE OF LACTIC ACID.
—OCCULT BLOOD.
B—Feces—OCCULT BLOOD.
3. Of kidney—HEMATURIA.
4. Of pancreas—STEATORRHEA.
5. Of uterus—**Uterine scrapings.**

CHLOROSIS.
1. Blood—ABSOLUTE AND DIFFERENTIAL COUNT.
—HEMOGLOBIN.

CHOLERA Asiatic.
Feces—**Bacteria.**

CONJUNCTIVITIS.
1. Discharges—BACTERIA.

CYSTITIS.
1. Urine—PUS.
—BACTERIA.
—CHEMICAL ANALYSIS.

DIABETES.
1. Urine—SUGAR.
—ACETONE.
—Diacetic acid.
—B-oxybutyric acid.

DIPHTHERIA.
1. Smear and Culture—BACTERIA.

EMPHYSEMA.
1. Blood—BLOOD-PICTURE—HYPEREOSINOPHILIA.

ENDOCARDITIS.
1. Blood—LEUKOCYTE COUNT.
—Bacteria.

FILARIASIS.
1. Blood—PARASITES.
—DIFFERENTIAL COUNT.

GLANDERS.
1. Discharge from nose and lesions—**Bacteria.**

GONORRHEA.
1. Discharge—BACTERIA.
2. Urine—PUS AND SHREDS.

HEART DISEASE—Chronic Valvular.
1. Sputum—HEART LESION CELLS.

HYPERACIDITY OF STOMACH—Gastrosuccorrhœa acida.
1. Stomach contents—HYPERCHLORHYDRIA.
—TRISEDIMENTATION OF VOMITUS.

HYDATID DISEASE.
1. Fluid from cyst, sputum, etc.—HOOKLETS.

INFLUENZA.
1. Sputum—BACTERIA.

INTESTINAL HELMINTHIASIS—See Animal Parasites.

LEAD POISONING.
1. Blood—**Blood-picture—Basophilic granular degeneration.**

LEPROSY.
1. Blood from nodules—**Bacteria.**

LEUKEMIA.
1. Blood—ABSOLUTE AND DIFFERENTIAL LEUKOCYTE COUNT.

LUNG ABSCESS AND GANGRENE.

1. Sputum—PUS CELLS.
   —ELASTIC TISSUE.
   —FRAGMENTS OF LUNG.
   —BACTERIA.

MALARIA.

1. Blood—SPECIAL STAIN FOR PLASMODIUM.

MEASLES.

1. Blood—DIFFERENTIAL LEUKOCYTE COUNT.

MENINGITIS.

1. Blood—ABSOLUTE AND DIFFERENTIAL LEUKOCYTE COUNT.
2. Cerebro-spinal fluid—BACTERIA.
   —DIFFERENTIAL CELL COUNT.

MYELOMATOSIS.

1. Urine—BENCE JONES'S PROTEIN.

NEPHRITIS.

1. Urine—CHEMICAL EXAMINATION—ALBUMIN.
   —MICROSCOPIC EXAMINATION—CASTS, etc.
2. Blood-pressure—

OTITIS MEDIA AND MASTOIDITIS.

1. Discharge—BACTERIOLOGIC EXAMINATION.

PARESIS.

1. **Blood—Wassermann.**
2. Cerebro-spinal fluid—ABSOLUTE AND DIFFERENTIAL LEUKOCYTE COUNT.
   —NOGUCHI TEST.

PNEUMONIA.

1. Sputum—BACTERIA.
2. Blood—DIFFERENTIAL LEUKOCYTE COUNT.
3. Urine—CHLORIDES.

PSEUDOLEUKEMIA—Hodgkin's disease.

1. Blood—ABSOLUTE AND DIFFERENTIAL LEUKOCYTE COUNT.

PYELITIS.

1. Urine—PUS.
   —BACTERIA.
   —OCCULT BLOOD.
   —Permeation test.

RABIES.
1. Brain tissue—**Negri bodies.**

SCARLATINA.
1. Blood—DIFFERENTIAL LEUKOCYTE COUNT.

SEPTIC INFECTIONS.
1. Blood—ABSOLUTE AND DIFFERENTIAL LEUKOCYTE COUNT.
—**Bacteriologic examination.**
2. Pus—BACTERIA.

SYPHILIS.
1. Primary lesion—Smear for specific bacteria.
2. Secondary lesion
3. Tertiary—
4. Tabes and G. P. I.

— Blood—**Wassermann.**
— Cerebro-spinal fluid—NOGUCHI.

TETANUS.
1. Discharge—**Smear for specific bacteria.**

TONSILLITIS.
1. Exudate—BACTERIOLOGIC EXAMINATION.
2. Blood—DIFFERENTIAL LEUKOCYTE COUNT.

TRICHINOSIS.
1. Blood—HYPEREOSINOPHILIA.
2. Feces—PARASITES **Adults or embryos.**
3. **Muscle tissue—Trichinella embryos encysted.**

TRYPANOSOMIASIS.
1. Blood—DIFFERENTIAL LEUKOCYTE COUNT.
—TRYPANOSOMES.
2. Cerebro-spinal fluid—TRYPANOSOMES.

TUBERCULOSIS.
1. Sputum—TUBERCLE BACILLI.
2. Blood—LYMPHOCYTOSIS.
3. **Tissue—Tubercles.**
4. Reaction—TUBERCULIN.

TYPHOID FEVER.
1. Blood—WIDAL—MACROSCOPIC.
—**Widal—Microscopic.**
—**Bacteria.**

TUMORS—in general.
1. Tissue—**Microscopic examination.**

VINCENT'S ANGINA.
1. Exudate—**Bacteriologic examination.**

URINALYSIS.

Report of Clinical Laboratory.

.....

.....

Physical Characteristics.		Quantitative Determinations.
Quantity in 24 hours.	c.cm. / oz.	Albumin: Heat, 10% Acetic Acid. / Heller's Nitric Acid. / Heat and Nitric Acid.
Color,		Glucose: Fehling's. / Phenylhydrazin. / Böttger's Bismuth.
Appearance,		
Odor,		
Sediment:—		
Mucus,		Indican,
Urates,		Skatol,
Phosphates,		Acetone,
Uric Acid,		Bile Pigments,
Pus,		Blood,
Specific Gravity,		Diazo,
Reaction,		Aceto-acetic Acid,
		B-Oxybutyric Acid,
		Drug Reactions,
		Cammidge Reaction,

Quantitative Determination.

Albumin (Esbach), (Purdy),
Glucose (Fehling's), (Fermentation),
Urea,
Ethereal Sulphates,
Chlorids,

Microscopic Examination.

(Centrifugated),		(Sedimented),
Casts.	Hyaline, / Granular, / Fatty, / Epithelial, / Blood, / Waxy, / Bacterial,	Erythrocytes, / Leukocytes, / Pus cells, / Epithelia, / Spermatazoa, / Bacteria, / Yeast Spores,
Cylindroids,		Trichomonides.
Mucus,		

Voided, A.M.....P.M..... Examined, A.M.....P.M.....

Examined by .....

SPUTUM EXAMINATION.

Report of Clinical Laboratory.

.....

.....

Physical Characteristics.

Quantity in 24 hours. } c.cm. / oz.

Color,

Odor,

Consistence,

Blood,

Character,

Serous,

Mucous,

Muco-purulent,

Purulent,

Casts,

Spirals,

Microscopic Examination.

Tubercle Bacilli,	Blood-Cells,
Micrococcus Lanceolatus,	Pus Cells,
Staphylococci,	"Heart Failure" Cells,
Streptococci,	Hematin Crystals,
Bacilli,	Elastic Fiber,
Actinomyces,	Curschmann's Spirals,
Yeast,	Casts,

Obtained ..... Examined .....

Examined by .....

BLOOD EXAMINATION.

Report of Clinical Laboratory.

.....

.....

General Examination.

Color,

Coagulability { Estimated,
Wright's,
Bogg's,

Viscosity,
Flow from Puncture,
Specific Gravity,

Erythrocytes { Thoma-Zeiss per c.mm.,
Estimated by Daland Hematokrit,
Percent.,

Hemoglobin { Fleischl's Hemoglobinometer,
Sahli's Hemoglobinometer,
Tallqvist's Scale,

Color Index,
Plaques,
Leukocytes,

Microscopic Examination.

Erythrocytes.

Color,
Polychromatophilia,
Granular Degeneration,
Poikilocytosis,
Macrocytes,
Microcytes,
Normoblasts,

Leukocytes.

Differential Count,
Number of cells counted,
Polymorphonuclears,
Large lymphocytes,
Small lymphocytes,
Eosinophiles,
Basophiles,
Myelocytes,
Parasites,
Bacteria,
Hematin Crystals,

Date .....

Examined by .....

GASTRO-ANALYSIS.

Report of Clinical Laboratory.

.....

.....

Vomitus,
Test meal

Composition:—

Ingested,
Extracted,
Amount Recovered,
Dilution,

PHYSICAL CHARACTERISTICS.

Sediment containing:—

Color,
Odor,
Consistency,
Reaction,

Blood,
Bile,
Mucus,

CHEMICAL DETERMINATIONS.

Total Acidity,
Free Hydrochloric Acid,
Combined " "
Lactic Acid,
Protealysis,

Butyric Acid,
Acetic Acid,
Blood,
Bile,

MICROSCOPIC EXAMINATION.

Starch Grains,
Meat Fiber,
Epithelia,
Erythrocytes,
Leukocytes,

Oppler-Boas Bacillus,
Sarcinæ,
Bacteria,
Necrotic Tissue,
Parasites,

Date ..... Examined .....

Examined by .....

EXAMINATION OF THE FECES.

Report of Clinical Laboratory.

.....

.....

Physical Characteristics.

Number of Stools in 24 hours.
Amount in 24 Hours.
Color,
Odor,
Consistence,
Blood,
Pus,
Mucus,
Parasites,
Crystals,
Calculi,
Connective tissue,
Muscle fiber,
Foreign bodies,

Chemical Examination.

Blood,
Hydrobilirubin (sublimate test),
Fermentation.
"Lost" Albumin,

Microscopic Examination.

Erythrocytes,
Pus cells,
Epithelia,
Crystals,
Meat Fiber,
Connective Tissue,
Starch Cells,
Free Fat,
Fatty acids,
Tubercle Bacillus,
Shiga Bacillus,
Comma Bacillus,
B. Aerogenes Capsulatus,
Amebæ,
Parasites.

Obtained ..... Examined .....

Examined by .....

BLOOD-PRESSURE DETERMINATIONS.

Clinical Report.

.....

.....

Apparatus

Width of Cuff cm.
Part examined,
Right,
Left,
Posture,
Pulse Rate,
Systolic mm.Hg.
Diastolic mm.Hg.
Pulse Pressure mm.Hg.
Mean Pressure mm.Hg.

Remarks.

Time of Day. A. M..... P. M.....

Date .....

Examined by .....

BLOOD-PRESSURE CHART.

A New Graphic Chart for Recording Blood-Pressure Observations.—This chart will be found very valuable in connection with serial studies of blood-pressure where it is desired

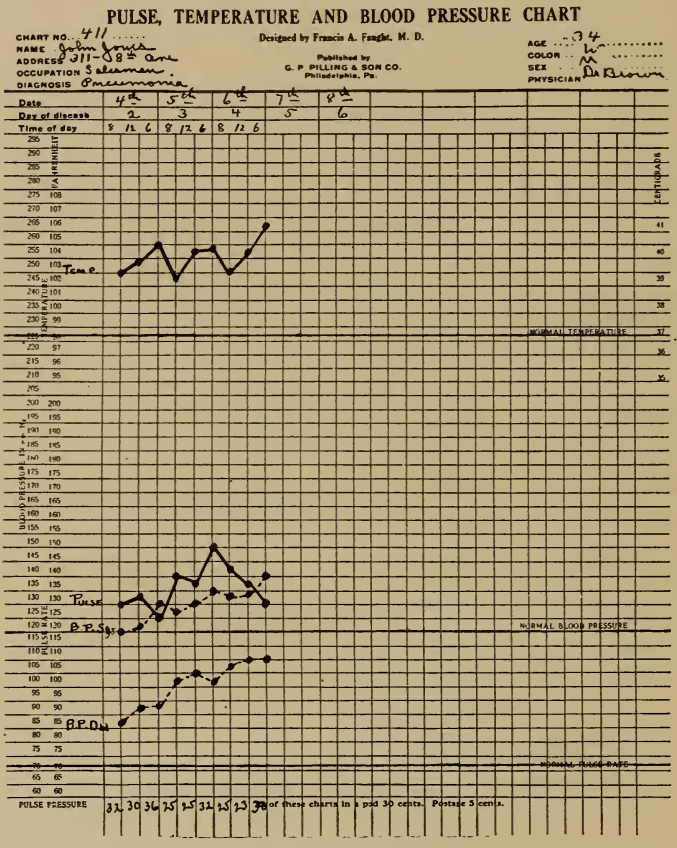


FIG. 57.—SPECIMEN OF A NEW GRAPHIC CHART FOR RECORDING BLOOD PRESSURE OBSERVATIONS, ARRANGED FOR DAILY, WEEKLY OR IRREGULAR OBSERVATION PERIODS. SPACES EQUAL 5 MILLIMETERS.

to follow from time to time its fluctuations. It is constructed along the lines of a temperature chart. The changes in blood-pressure can be readily followed, and the effect of treatment more certainly demonstrated.

Bang's Table of Reduction Equivalents.

(Webster: "Diagnostic Methods," 1912.)

Cubic centimeters of hydroxylamin solution.	Milligrams of sugar.	Cubic centimeters of hydroxylamin solution.	Milligrams of sugar.
0.75	60.0	25.50	23.5
1.00	59.4	26.00	22.9
1.50	58.4	26.50	22.3
2.00	57.3	27.00	21.8
2.50	56.2	27.50	21.2
3.00	55.0	28.00	20.7
3.50	54.3	28.50	20.1
4.00	53.4	29.00	19.6
4.50	52.6	29.50	19.1
5.00	51.6	30.00	18.6
5.50	50.7	30.50	18.0
6.00	49.8	31.00	17.5
6.50	48.9	31.50	17.0
7.00	48.0	32.00	16.5
7.50	47.2	32.50	15.9
8.00	46.3	33.00	15.4
8.50	45.5	33.50	14.9
9.00	44.7	34.00	14.4
9.50	44.0	34.50	13.9
10.00	43.3	35.00	13.4
10.50	42.5	35.50	12.9
11.00	41.8	36.00	12.4
11.50	41.1	36.50	11.9
12.00	40.4	37.00	11.4
12.50	39.7	37.50	10.9
13.00	39.0	38.00	10.4
13.50	38.3	38.50	9.9
14.00	37.7	39.00	9.4
14.50	37.1	39.50	9.0
15.00	36.4	40.00	8.5
15.50	35.8	40.50	8.1
16.00	35.1	41.00	7.6
16.50	34.5	41.50	7.2
17.00	33.9	42.00	6.7
17.50	33.3	42.50	6.3
18.00	32.6	43.00	5.8
18.50	32.0	43.50	5.4
19.00	31.4	44.00	4.9
19.50	30.8	44.50	4.5
20.00	30.2	45.00	4.1
20.50	29.6	45.50	3.7
21.00	29.0	46.00	3.3
21.50	28.3	46.50	2.9
22.00	27.7	47.00	2.5
22.50	27.1	47.50	2.1
23.00	26.5	48.00	1.7
23.50	25.8	48.50	1.3
24.00	25.2	49.00	0.9
24.50	24.6	49.50	0.5
25.00	24.1	50.00	0.0

For every 0.1 c.c. hydroxylamin solution used more than is given in the table, subtract 0.1 mg. if the reading be between 49 and 15, while if it be between 15 and 1 subtract 0.2 mg.

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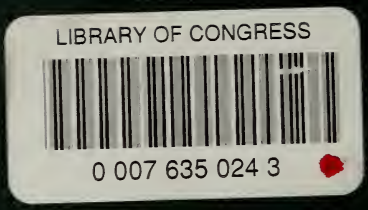
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